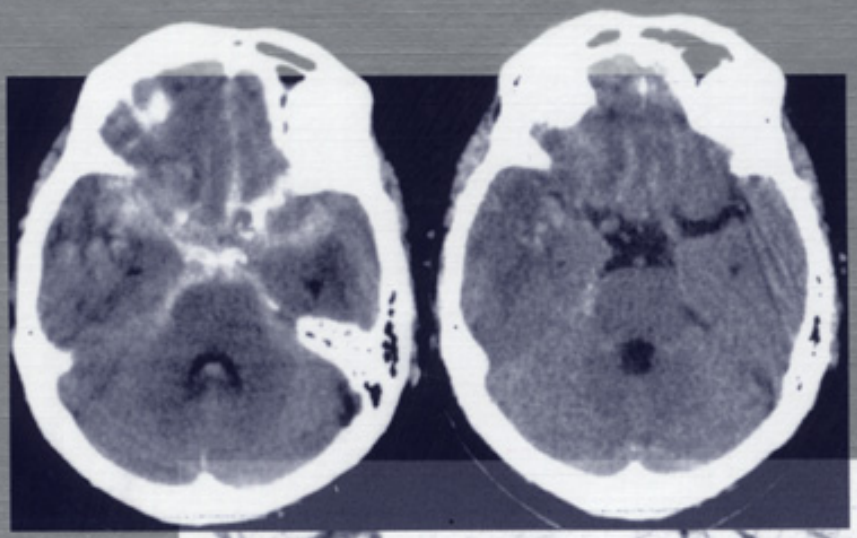
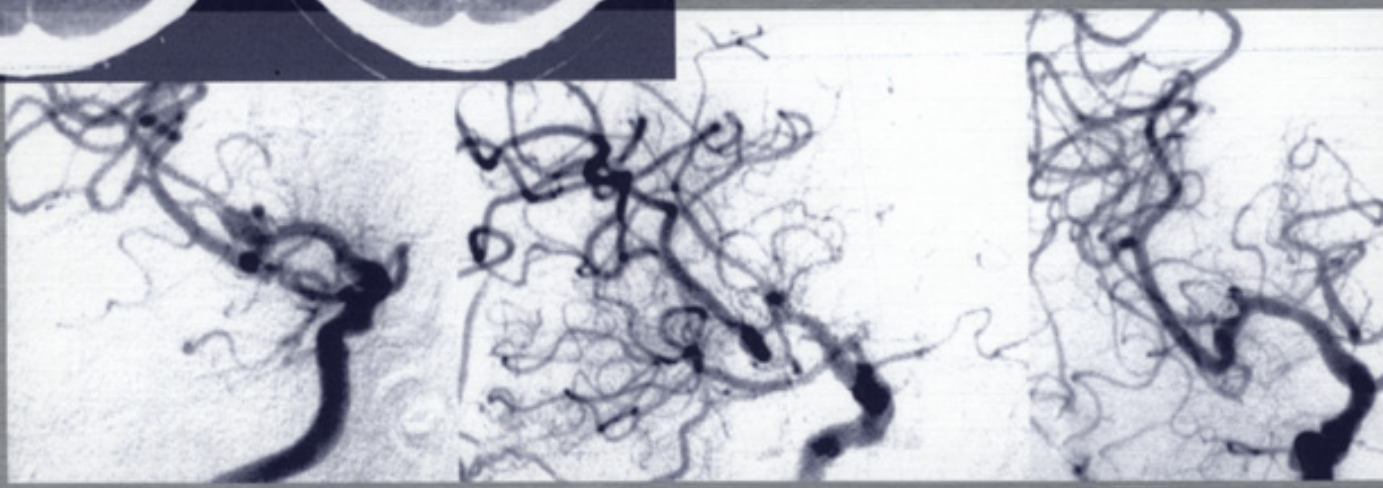
Cerebral Vasospasm

Advances in Research and Treatment

R. Loch Macdonald





Thieme

Cerebral Vasospasm

Advances in Research and Treatment

Cerebral Vasospasm
Advances in Research and Treatment

R. Loch Macdonald, M.D., Ph.D.

Professor
Department of Neurosurgery
University of Chicago Medical Center
Chicago, IL

Thieme
New York • Stuttgart

Thieme Medical Publishers, Inc.
333 Seventh Ave.
New York, NY 10001

Assistant Editor: Jennifer Berger
Editor: Timothy Hiscock
Vice President, Production and Electronic Publishing:
Anne T. Vinnicombe
Production Editor: Print Matters, Inc.
Marketing Director: Phyllis Gold
Sales Manager: Ross Lumpkin
Chief Financial Officer: Peter van Woerden
President: Brian D. Scanlan
Compositor: Compset, Inc.
Printer: Sheridan Books

Library of Congress Cataloging-in-Publication Data

International Conference on Cerebral Vasospasm (8th : 2003 : Chicago, Ill.)
Cerebral vasospasm : advances in research and treatment / [edited by] R. Loch MacDonald.
p. ; cm.
Includes bibliographical references and index.
ISBN 1-58890-283-8 (TMP : HC)—ISBN 3-13-140061-7 (HC)—ISBN 3-13-130781-1 (GTV)
1. Cerebrovascular spasm—Congresses. I. Macdonald, R. Loch (Robert Loch) II. Title.
[DNLM: 1. Vasospasm, Intracranial—Congresses. 2. Subarachnoid
Hemorrhage—complications—Congresses. WL 355 I601 ca 2005]
RC388.5.I425 2003
616.8'1—dc22

2004053710

Important note: Medical knowledge is ever-changing. As new research and clinical experience broaden our knowledge, changes in treatment and drug therapy may be required. The authors and editors of the material herein have consulted sources believed to be reliable in their efforts to provide information that is complete and in accord with the standards accepted at the time of publication. However, in the view of the possibility of human error by the authors, editors, or publisher of the work herein or changes in medical knowledge, neither the authors, editors, or publisher, nor any other party who has been involved in the preparation of this work, warrants that the information contained herein is in every respect accurate or complete, and they are not responsible for any errors or omissions or for the results obtained from use of such information. Readers are encouraged to confirm the information contained herein with other sources. For example, readers are advised to check the product information sheet included in the package of each drug they plan to administer to be certain that the information contained in this publication is accurate and that changes have not been made in the recommended dose or in the contraindications for administration. This recommendation is of particular importance in connection with new or infrequently used drugs.

Printed in the United States

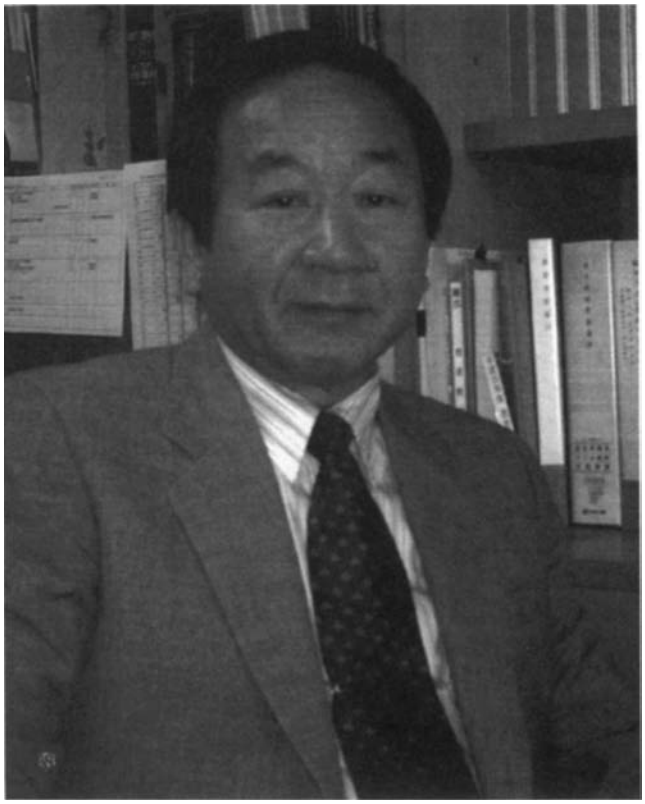
5 4 3 2 1

TMP ISBN 1-58890-283-8
GTV ISBN 3-13-130781-1

Dedication

This book is dedicated to the physicians, nurses, and health care professionals who strive to reduce the burden of cerebral vasospasm in those stricken by subarachnoid hemorrhage. In addition, it is dedicated to the patients and their families who, after the hemorrhage, must cope with the agony of worrying for days in the intensive care unit: Will they or their loved one be stricken by the dreaded second stroke? Then, the patients and families must handle—and they often do so remarkably well—the months and years of a life frequently irreversibly altered by subarachnoid hemorrhage and vasospasm.

I also dedicate this work to the honored guests of the Eighth International Conference on Cerebral Vasospasm, Drs. Tomio Ohta (below right) and Shigeharu Suzuki (below left). Dr. Ohta is professor emeritus of the Department of Neurosurgery at Osaka Medical College. He conducted and published some of the earliest work identifying hemoglobin as a key contributor to vasospasm. Dr. Suzuki is chairman, Department of Neurosurgery at Hirosaka University School of Medicine. Among many contributions to the field of vasospasm, Dr. Suzuki has been a driving force in the testing of the hypothesis that microcirculatory changes are a critical contributor to the syndrome of delayed ischemic neurological deficits after subarachnoid hemorrhage. Both of these outstanding physicians, scientists, and men have been in a way responsible for paradigm shifts in thinking about cerebral vasospasm and delayed ischemia. I believe this volume shows they have stimulated thought and research to continue such advancements in the field.



Shigeharu Suzuki, M.D.

Tomio Ohta, M.D.

Contents

Section VI. Clinical—Doppler and Imaging

Section VII. Clinical—Medical Aspects

Preface

Vasospasm remains a substantial cause of morbidity and mortality, usually from a ruptured intracranial aneurysm, in those patients who survive the initial subarachnoid hemorrhage. This book gives an update on the etiology, pathogenesis, epidemiology, clinical and radiological investigation, and treatment of delayed ischemic neurological deficits and vasospasm. Physicians in the fields of neurosurgery, neuroradiology, neurointensive care, and other allied areas will benefit in the management of their patients from perusal of this text.

Introduction

This book contains papers presented at the Eighth International Conference on Cerebral Vasospasm that was held in Chicago from July 9 to 12, 2003. There were 120 attendees, almost seven times as many as attended the first of these conferences. The proceedings of each of the conferences have been published.[1–7] Perusal of their contents shows the remarkable progress made in this area.

The first of the "vasospasm" meetings was convened November 10 and 11, 1972, in Jackson, Mississippi. There were 18 attendees. Drs. Robert R. Smith and John T. Robertson organized the meeting, and the honored guest was, fittingly for the first meeting, Dr. Francis A. Echlin. Attention to the field of cerebral vasospasm grew among neurosurgeons on every continent, with particular interest in Japan and Europe. Annual conferences have been held in Japan for some years now, and undoubtedly there have been others. The second meeting, called an international workshop, was the work initially of Dr. A. J. M. van der Werf. The program committee consisted of Dr. R. H. Wilkins as chairman, Dr. van der Werf as secretary, and Drs. S. Ishii, S. J. Peerless, and L. Symon as members. Dr. C. Miller Fisher was honored guest of the meeting that was held in Amsterdam in 1979.

The third meeting was in Charlottesville, Virginia, in 1987. Dr. Neal Kassell was the chairman, and Dr. Dennis Vollmer was secretary. It was held at the University of Virginia April 29 to May 1,1987. Dr. Keiji Sano was honored guest, and Dr. Charles G. Drake was honorary president.

Dr. Keiji Sano and the University of Tokyo hosted the fourth meeting in Tokyo May 15 to 18, 1990. The honored guest was Dr. Bryce Weir, Dr. Lindsay Simon was honorary president, Dr. Keji Sano was president, Drs. Kintomo Takakura and Neal F. Kassell were vice presidents, and Drs. Isamu Saito and Tomio Sasaki were secretaries.

The fifth international conference was held in Edmonton and Jasper, Alberta, Canada from May 17 to 21, 1993. Dr. Bryce Weir was president, and Dr. Neal Kassell was honored guest. Dr. Max Findlay was secretary treasurer and was editor of the proceedings. The sixth conference was in Sydney, Australia, from May 15 to 17, 1997. Dr. Nicholas Dorsch was president, and Dr. Robert R. Smith was honored guest, and the proceedings were dedicated to him. Dr. Smith recently passed away. His contributions to the field are many. He was the organizer of the first conference and, as such, started it all. Dr. Smith was an early proponent of balloon angioplasty in North America. He recognized the resistance of vasospastic arteries to vasodilators and proposed a theory regarding the role of novel contractile mechanisms in the pathogenesis of vasospasm. The conference met for the seventh time in Interlaken, Switzerland, under the presidency of Dr. Rolf Seiler. The meeting was held from June 17 to 21, 2000. Dr. Helge Nornes was the honored guest.

I organized the eighth meeting in Chicago and served as president. The honored guests were Drs. Tomio Ohta and Shigeharu Suzuki. The secretary was Dr. John Zhang, and other members of the organizing committee were Drs. Ryszard Pluta, Mario Zuccarello, and Joseph Clark. I obtained financial support from industry and philanthropic sources. The participants themselves, however, made the meeting a success, and I am eternally grateful to all of them for presenting their work and agreeing to publish it in these pages. I feel somewhat insignificant to be a name now listed almost by default amongst the giants who have driven the vasospasm field.

I have extensively edited the manuscripts for this book and have attempted to correct grammar and spelling in order to make this volume one that can be read quickly and easily, leaving the reader with an idea of the state of the field of vasospasm research. I would like to thank the companies that supported the meeting, the members of the scientific planning committee, and the attendees for making it possible. I also thank Marlene Goldberg, Lydia Johns, and Chris Reilly for their help with the meeting.

R. Loch Macdonald, M.D., *Ph.D.*
Chicago, Illinois

REFERENCES

1. Smith RR, Robertson JT, eds. Subarachnoid Hemorrhage and Cerebrovascular Spasm. Springfield, IL: CC Thomas; 1975
2. Wilkins RH, ed. Cerebral Arterial Spasm. Proceedings of the Second International Workshop, Amsterdam, The Netherlands, 1979. Baltimore, MD: Williams and Wilkins; 1980
3. Wilkins RH, ed. Cerebral Vasospasm. New York, NY: Raven Press; 1988
4. Sano K, Takakura K, Kassell NF, Sasaki T, eds. Cerebral Vasospasm. Proceedings of the IVth International Conference on Cerebral Vasospasm, Tokyo, 1990. Tokyo, Japan: University of Tokyo Press; 1990
5. Findlay JM, ed. Cerebral Vasospasm. Proceedings of the Vth International Conference on Cerebral Vasospasm, Edmonton and Jasper, Alberta, Canada, May 17-21, 1993. Amsterdam, The Netherlands: Elsevier; 1993
6. Dorsch NWC, ed. Cerebral Vasospasm VI. Proceedings of the VIth International Conference on Cerebral Vasospasm. Sydney, Australia: Oslington Consulting; 1999
7. Seiler RW, Steiger HJ, eds. Cerebral Vasospasm. Acta Neurochir (Suppl 77); Wien, Springer Verlag; 2001

Contributors

Mayowa Agbaje-Williams, B.A.
Departments of Surgery and Neurosurgery
University of Chicago
Chicago, IL

Yasuo Aihara, M.D., Ph.D.
Department of Neurosurgery
University of Chicago Medical Center
Chicago, IL

Hiroyuki Akagawa, M.D.
Department of Neurosurgery
Tokyo Women's Medical University
Tokyo, Japan

Alessio Albanese, M.D.
Institute of Neurosurgery
Catholic University School of Medicine
Rome, Italy

Richard Aldrich, Ph.D.
Department of Neurosurgery
Stanford University School of Medicine
Stanford, CA

Pippa Al-Rawi, B. Sc.
Academic Neurosurgery Unit
Addenbrooke's Hospital
Cambridge, UK

Norberto Andaluz, M.D.
Cerebrovascular Fellow
Department of Neurosurgery
University of Cincinnati
Cincinnati, OH

Robert Andres, M.D.
Oberarzt Neurochirurgie
Inselspital Bern
Universität Bern
Bern, Switzerland

Kazuya Aoki, M.D.
Assistant Professor
Department of Neurosurgery
Toho University School of Medicine
Tokyo, Japan

Yoshikazu Arai, M.D.
Department of Neurosurgery
Fukui Medical University
Fukui, Japan

Naoki Asami, M.D.
Assistant Professor
Department of Neurosurgery
Miyazaki Medical College
Miyazaki, Japan

Lorence Barth, M.D.
Oberarzt Neurochirurgie
Inselspital Bern
Universität Bern
Bern, Switzerland

Jürgen Beck, M.D.
Department of Neurosurgery
Johann Wolfgang Goethe University
Frankfurt, Germany

Leona Beecroft, R.G.N., B. Sc.
Leeds General Infirmary
Leeds, UK

Susan J. Biehle, Ph.D.
Department of Neurology
University of Cincinnati
Cincinnati, OH

Dieter–Karsten Böker, M.D.
Professor
Department of Neurosurgery
University Hospital Giessen
Giessen, Germany

Robert Brenner, Ph.D.
Stanford University School of Medicine
Stanford, CA

Thomas A. D. Cadoux-Hudson, M.D., Ph.D.
Department of Biochemistry
MRC Collaborative Center
University of Oxford
Oxford, UK

Tarkan Calisaneller, M.D.
Department of Neurological Surgery
University of Virginia
Charlottesville, VA

Pak Chan, Ph.D.
Department of Neurosurgery
Stanford University School of Medicine
Stanford, CA

John C. Clark, Ph.D.
Wolfson Brain Imaging Centre
University of Cambridge
Addenbrooke's Hospital
Cambridge, UK

Joseph F. Clark, Ph.D.
Associate Professor
Department of Neurology
University of Cincinnati
Cincinnati, OH

Wendy Cohen, M.D.
Departments of Neurological Surgery and Radiology
University of Washington
Harborview Medical Center
Seattle, WA

Reinaldo Correa, M.D.
Department of Neurological Surgery
University of Washington
Harborview Medical Center
Seattle, WA

Marek Czosnyka, Ph.D.
Academic Neurosurgery Unit
University of Cambridge
Addenbrooke's Hospital
Cambridge, UK

Isao Date, M.D.
Department of Neurological Surgery
Okayama University Graduate School of Medicine and Dentistry
Okayama, Japan

Charles H. G. Davis, F.R.C.S.
University of Bristol
Royal Preston Hospital
Bristol, UK

Wolfgang Deinsberger, M.D.
Department of Surgery
University Hospital Giessen
Giessen, Germany

Edgar Dettmann, M.D.
Department of Neurosurgery
Johann Wolfgang Goethe University
Frankfurt, Germany

Federico Di Rocco, M.D.
Resident
Institute of Neurosurgery
Catholic University School of Medicine
Rome, Italy

Robert L. Dodd, M.D., Ph.D.
Department of Neurosurgery
Stanford University School of Medicine
Stanford, CA

Aaron S. Dumont, M.D.
Department of Neurological Surgery
University of Virginia Health Sciences Center
Charlottesville, VA

Lars Edvinsson, M.D., Ph.D.
Professor
Department of Internal Medicine
Lund University Hospital
Lund, Sweden

Tülen Erden, M.D.
Istanbul University
Istanbul Medical School
Istanbul, Turkey

Figen Esen, M.D.
Istanbul University
Istanbul Medical School
Istanbul, Turkey

Javier Fandino, M.D.
Department of Neurosurgery
University Hospital Bern
Bern, Switzerland

Jeffrey Farkas, M.D.
Department of Radiology
Maimonides Medical Center
Brooklyn, NY

J. Max Findlay, M.D., Ph.D.
Clinical Professor
Department of Surgery
University of Alberta Hospital
Edmonton, AB

Edward R. Flotte, M.D.
Assistant Professor
University of Mississippi
University of Mississippi Medical Center
Jackson, MS

Satoshi Fujii, M.D.
Division of Neurosurgery
Yokohama Stroke and Brain Center
Yokohama, Japan

Naoko Fujimura, M.D., Ph.D.
Assistant Professor
Department of Neurosurgery
Kurume University School of Medicine
Kurume, Japan

Hiroaki Fujiwara, M.D.
Department of Neurosurgery
Yamamoto Daisan Hospital
Osaka, Japan

Hironori Furukawa, M.D.
Department of Neurosurgery
Toho University School of Medicine
Tokyo, Japan

Jo Geldard, R.G.N.Dip.
Department of Neurosciences
Leeds General Infirmary
Leeds University
Leeds, England, UK

Cheryl E. Geriepy, M.D.
University of Michigan Medical School
Ann Arbor, MI

Yoshikazu Goto, M.D.
Emergency and Critical Care Medicine
Kanazawa University School of Medicine
Kanazawa, Japan

Tomokazu Goya, M.D., Ph.D.
Director
Department of Neurosurgery
Junwakai Memorial Hospital
Miyazaki, Japan

N. T. Gurusinghe
Department of Neurological Surgery
Royal Preston Hospital
University of Bristol
Bristol, UK

Dong-Han Han, M.D.
Department of Neurosurgery
Chonbuk National University Hospital
Chonbuk, Korea

Yuji Handa, M.D., Ph.D.
Department of Neurosurgery
Fukui Medical University
Fukui, Japan

Jacob Hansen-Schwartz, M.D., Ph.D.
Research Fellow
Department of Clinical Experimental Research
Glostrup University Hospital
Glostrup, Denmark

Judith Harvey-White, B.S.
Laboratory of Physiologic Studies
National Institute on Alcohol Abuse and Alcoholism
National Institutes of Health
Bethesda, MD

Yasuhiro Hashiba, M.D.
Assistant Professor
Department of Neurosurgery
Gunma University School of Medicine
Gunma, Japan

Morito Hayashi, M.D.
Department of Neurosurgery
Toho University School of Medicine
Tokyo, Japan

Yasuhiko Hayashi, M.D.
Department of Neurosurgery
Kanazawa University School of Medicine
Kanazawa, Japan

LiYun He, Ph.D.
Department of Neurology
Beijing University of Chinese Medicine
DongZhiMen Hospital
Beijing, China

Sabine Heiland, Ph.D.
Department of Neuroradiology
University of Heidelberg
Mannheim, Germany

Elizabeth M. A. Hensor, Ph.D.
Department of Rheumatology
University of Leeds
Leeds, UK

Masaru Hirohata, M.D.
Assistant Professor
Department of Neurosurgery
Kurume University School of Medicine
Kurume, Japan

Tomohito Hishikawa, M.D.
Resident
Department of Neurological Surgery
Okayama University Graduate School of Medicine and Dentistry
Okayama, Japan

Natalie Løvland Hoel, B.S.
Research Assistant
Department of Clinical Experimental Research
Glostrup University Hospital
Glostrup, Denmark

Tomokatsu Hori, M.D.
Department of Neurosurgery
Tokyo Women's Medical University
Tokyo, Japan

Peter Horn, M.D.
Department of Neurosurgery
University of Heidelberg
Mannheim, Germany

Monika Hügens-Penzel, M.D.
Department of Neuroradiology
University Hospital Giessen
Giessen, Germany

C. David Hunt, M.D.
Director
Department of Surgery
Division of Neurosurgery
Maimonides Medical Center
Brooklyn, NY

Linda P. Hunt, Ph.D.
University of Bristol
Royal Preston Hospital
Bristol, UK

Hideaki Imai, M.D., Ph.D.
Department of Neurosurgery
Gunma University School of Medicine
Gunma, Japan

Hideo Inaba, M.D.
Department of Neurosurgery
Kanazawa University School of Medicine
Kanazawa, Japan

Ken Inweregbu, M.B.Ch.B., B.Sc, F.R.C.A.
Department of Anaesthetics
Leeds General Infirmary
Leeds, UK

Masaki Ishida, M.D., Ph.D.
Department of Neurosurgery
Fukui Medical University
Fukui, Japan

Tatsuhiro Ishida, Ph.D.
Department of Pharmacokinetics and Biopharmaceutics
University of Tokushima
Tokushima, Japan

Hisamasa Ishii, M.D.
Department of Neurosurgery
Fukui Medical University
Fukui, Japan

Eiji Isotani, M.D., Ph.D.
Department of Neurosurgery
Tokyo Medical and Dental University
Tokyo, Japan

Brian A. Iuliano, M.D.
Surgical Neurology Branch
National Institutes of Neurological Disorders and Stroke
National Institutes of Health
Bethesda, MD

Satoshi Iwabuchi, M.D.
Assistant Professor
Department of Neurosurgery
Toho University School of Medicine
Tokyo, Japan

Anthony Jabre, M.D.
Department of Neurosurgery
Boston University Medical Center
Boston, MA

Vikram D. Jadhav, M.B.B.S.
Department of Neurosurgery
Boston University Medical Center
Boston, MA

Babak S. Jahromi, M.D., Ph.D.
Resident
Department of Surgery
University of Toronto
Toronto, ON

William D. Johnson, Ph.D.
Associate Professor
Department of Epidemiology
University of Mississippi Medical Center
Jackson, MS

Carla S. Jung, M.D.
Surgical Neurology Branch
National Institutes of Neurological Disorders and Stroke
National Institutes of Health
Bethesda, MD

Kenji Kanamaru, M.D., Ph.D.
Department of Neurosurgery
Suzuka Kaisei Hospital
Sukuka, Japan

Neal F. Kassell, M.D.
Department of Neurological Surgery
University of Virginia
Charlottesville, VA

Stefanie Kästner, M.D.
Department of Neurosurgery
University Hospital Giessen
Giessen, Germany

Hidetoshi Kasuya, M.D.
Assistant Professor
Department of Neurosurgery
Tokyo Women's Medical University
Tokyo, Japan

Yoichi Katayama, M.D., Ph.D.
Professor
Department of Neurological Surgery
Nihon University School of Medicine
Tokyo, Japan

Tatsuro Kawamata, M.D., Ph.D.
Assistant Professor
Department of Neurological Surgery
Nihon University School of Medicine
Tokyo, Japan

Hirokazu Kawano, M.D., Ph.D.
Associate Director
Department of Neurosurgery
Junwakai Memorial Hospital
Miyazaki, Japan

Shinya Kida, M.D.
Department of Neurosurgery
Kanazawa University School of Medicine
Kanazawa, Japan

Chul-Jin Kim, M.D., Ph.D.
Professor
Department of Neurosurgery
Chonbuk National University Hospital
Chonbuk, Korea

Hitoshi Kimura, M.D.
Department of Neurosurgery
Toho University School of Medicine
Tokyo, Japan

Talat Kiris, M.D.
Associate Professor
Department of Neurosurgery
Istanbul Medical School
Istanbul University
Istanbul, Turkey

Peter J. Kirkpatrick, M.B., Ch.B., F.R.C.S. (S.N.)
Consultant Neurosurgeon
Department of Neurosurgery
University of Cambridge
Addenbrooke's Hospital
Cambridge, UK

Ryuhei Kitai, M.D.
Department of Neurosurgery
Toho University School of Medicine
Tokyo, Japan

Paul Klimo Jr., M.D., M.P.H.
Resident
Department of Neurosurgery
University of Utah
Salt Lake City, UT

Namio Kodama, M.D.
Professor
Chair
Department of Neurosurgery
Fukushima Medical University
Fukushima, Japan

Masayo Koide, M.S.
Lecturer
Department of Neurosurgery
Hamamatsu University School of Medicine
Shizuoka, Japan

Jun Kojima, Ph.D.
Department of Neurological Surgery
Nihon University School of Medicine
Tokyo, Japan

Tadashi Kojima, M.D., Ph.D.
Department of Neurosurgery
Mie Prefectural General Medical Center
Yokkaichi, Japan

Yutaka Konno, M.D.
Instructor
Department of Neurosurgery
Fukushima Medical University
Fukushima, Japan

Toshihiko Kubota, M.D., Ph.D.
Fukui, Japan

Gen Kusaka, M.D.
Department of Neurosurgery
University of Mississippi Medical Center
Jackson, MS

Yong-Geun Kwak, M.D.
Department of Neurosurgery
Chonbuk National University Hospital
Chonbuk, Korea

Bettina Lange, M.D.
Department of Neurosurgery
Johann Wolfgang Goethe University
Frankfurt, Germany

Kevin S. Lee, Ph.D.
Department of Neuroscience
University of Virginia
Charlottesville, VA

Tony Jer-Fu Lee, Ph.D.
Professor
Department of Pharmacology
Southern Illinois University School of Medicine
Springfield, IL

David H. Lewis, M.D.
Department of Radiology
University of Washington
Harborview Medical Center
Seattle, WA

Alakandy M. Likhith, F.R.C.S.
University of Bristol
Royal Preston Hospital
Bristol, UK

Chih-Lung Lin, M.D.
Assistant Professor
Department of Neurosurgery
Kaohsiung Medical University
Kaohsiung, Taiwan

Melissa A. Lyons, Ph.D.
Department of Neurology
University of Cincinnati
Cincinnati, OH

Richard H. Lye, Ph.D.
University of Bristol
Royal Preston Hospital
Bristol, UK

R. Loch Macdonald, M.D., Ph.D.
Professor
Department of Neurosurgery
University of Chicago Medical Center
Chicago, IL

Giulio Maira, M.D.
Professor
Chair
Institute of Neurosurgery
Catholic University School of Medicine
Rome, Italy

Enrico Marchese, M.D.
Assistant Professor
Institute of Neurosurgery
Catholic University School of Medicine
Rome, Italy

Masato Matsumoto, M.D.
Assistant Professor
Department of Neurosurgery
Fukushima Medical University
Fukushima, Japan

Cindy Mayer, M.D.
Department of Radiology
University of Washington
Harborview Medical Center
Seattle, WA

Toshinari Meguro, M.D.
Research Associate
Department of Neurosurgery
University of Mississippi Medical Center
Jackson, MS

Aminidav Mendelowitsch, M.D.
Department of Neurosurgery
University of Basel
Basel, Switzerland

David K. Menon, M.B.B.S, M.D., Ph.D, F.R.C.P., F.R.C.A., F.Med.Sci.
Professor
Department of Anaesthesia
University of Cambridge
Addenbrooke's Hospital
Cambridge, UK

Heiko Mewes, M.D.
Department of Neurosurgery
University Hospital Giessen
Giessen, Germany

Pawan S. **Minhas,** M.D.
Academic Neurosurgery and Wolfson Brain Imaging Centre
University of Cambridge
Cambridge, UK

Ayako Mitate, M.D.
Department of Pharmacology
University of Shizuoka
Shizuoka, Japan

Tatsuro Mori, M.D., Ph.D.
Department of Neurological Surgery
Nihon University School of Medicine
Tokyo, Japan

Takuzo Moriyama, M.D., Ph.D.
Assistant Professor
Department of Neurosurgery
Miyazaki Medical College
Miyazaki, Japan

Nobuhiro Moro, M.D.
Department of Neurological Surgery
Nihon University School of Medicine
Tokyo, Japan

Elke Munch, M.D.
Ruprecht Kurts Universitat Heidelberg
Heidelberg, Germany

Hiroyuki Muramatsu, M.D.
Department of Neurosurgery
Fukushima Medical University
Fukushima, Japan

Masatoshi Muramatsu, M.D., Ph.D.
Department of Neurosurgery
Yao Tokushuukai Hospital
Osaka, Japan

Go **Nakagawa,** M.D.
Department of Neurosurgery
Toho University School of Medicine
Tokyo, Japan

Takao Nakagawa, M.D.
Department of Neurosurgery
Toho University
Tokyo, Japan

Shinichi Nakano, M.D., Ph.D.
Associate Professor
Department of Neurosurgery
Miyazaki Medical College
Miyazaki, Japan

Koichi Nakayama, Ph.D.
Professor
Department of Pharmacology
University of Shizuoka
Shziuoka, Japan

Shinsuke Nakayama, M.D., D.Phil.
Professor
Department of Cell Physiology
Nagoya University Medical School
Nagoya, Japan

Anil Nanda, M.D.
Professor
Chair
Department of Neurosurgery
Louisiana State University Health Sciences Center
Shreveport, LA

David W. Newell, M.D.
Professor
Department of Neurological Surgery
Harborview Medical Center
University of Washington
Seattle, WA

Sumbele Ngone, M.D.
Department of Neurosurgery
Johann Wolfgang Goethe University
Frankfurt, Germany

Elena Nikitina, Ph.D.
Departments of Surgery and Neurosurgery
University of Chicago
Chicago, IL

Mitsuhisa Nishiguchi, M.D.
Department of Neurological Surgery
Okayama University Graduate School of
Medicine and Dentistry
Okayama, Japan

Shinsaku Nishio, M.D.
Department of Neurological Surgery
Kagawa Prefectural Central Hospital
Kagawa, Japan

Shigeru Nishizawa, M.D., Ph.D.
Professor
Vice-Chair
Department of Neurosurgery
Hamamatsu University School of Medicine
Shizuoka, Japan

Yuichiro Nonaka, M.D.
Department of Neurosurgery
Hamamatsu University School of Medicine
Shizuoka, Japan

Yoshitsugu Nuki, M.D., Ph.D.
Assistant Professor
Department of Neurosurgery
Miyazaki Medical College
Miyazaki, Japan

Kazuo Obara, Ph.D.
Assistant Professor
Department of Pharmacology
University of Shizuoka
Shizuoka, Japan

Matthias F. Oertel, M.D.
Assistant Professor
Department of Neurosurgery
University Hospital Giessen
Giessen, Germany

Hiroki Ohkuma, M.D.
Department of Neurosurgery
Hirosaki University of Medicine
Hirosaki, Japan

Tomio Ohta, M.D., Ph.D.
Director
Tominaga Neurosurgical Hospital
Osaka, Japan

Masahiro Oinuma, M.D.
Instructor
Department of Neurosurgery
Fukushima Medical University
Fukushima, Japan

Edward H. Oldfield, M.D.
National Institutes of Health
Bethesda, MD

Bülent Omay, M.D.
Resident
Istanbul University
Istanbul Medical School
Istanbul, Turkey

Hideaki Onda, M.D.
Department of Neurosurgery
Tokyo Women's Medical University
Tokyo, Japan

Shigeki Ono, M.D.
Assistant of Neurological Surgery
Department of Neurological Surgery
Okayama University Graduate School of Medicine and Dentistry
Okayama, Japan

Virginia L. Overman, M.H.S.
Research Associate
Department of Neurosurgery
University of Mississippi Medical Center
Jackson, MS

Christoph Ozdoba, M.D.
Privatdozent
Department of Neuroradiology
Inselspital Bern
University of Bern
Bern, Switzerland

Andrew J. Patterson, M.D., Ph.D.
Assistant Professor of Anesthesia
Stanford University School of Medicine
Stanford, CA

Luigi Pentimalli, M.D.
Institute of Neurosurgery
Catholic University School of Medicine
Rome, Italy

Eddie Perkins, M.S.
Research Associate
Department of Neurosurgery
University of Mississippi Medical Center
Jackson, MS

John D. Pickard, M.D.
Professor
Academic Department of Neurosurgery
University of Cambridge
Addenbrooke's Hospital
Cambridge, UK

Sudakar Pipavath, M.D.
Departments of Neurological Surgery and Radiology
Harborview Medical Center
University of Washington
Harborview Medical Center
Seattle, WA

Michael H. Piper, M.D.
Department of Physiology
University of Giessen
Giessen, Germany

Ryszard M. Pluta, M.D., Ph.D.
Surgical Neurology Branch, NINDS
National Institutes of Health
Bethesda, MD

Sven Poli, M.D.
Department of Neurosurgery
University Hospital Mannheim
University of Heidelberg
Mannheim, Germany

Charles J. Prestigiacomo, M.D.
Assistant Professor
Departments of Neurological Surgery and Radiology
UMDNJ-Robert Wood Johnson Medical School
Newark, NJ

Gail J. Pyne-Geithman, D.Phil.
Department of Neurology
University of Cincinnati
Vontz Center for Molecular Studies
Cincinnati, OH

Audrey C. Quinn, M.B.Ch.B., F.F.A.R.C.S.I.
Consultant Anaesthetist
Department of Anaesthesia
Leeds General Infirmary
Leeds, UK

Andreas Raabe, M.D.
Associate Professor
Department of Neurosurgery
Johann Wolfgang Goethe University
Frankfurt am Main, Germany

Robert M. Rapoport, Ph.D.
Associate Professor
Department of Pharmacology and Cell Biophysics
University of Cincinnati College of Medicine
Cincinnati, OH

Frank A. Rasulo, M.D.
Consultant Anaesthetist–Intensivist
Department of Anaesthesia and Intensive Care
Spedali Civili University Hospital of Brescia
Brescia, Italy

Michael Reinert, M.D.
Department of Neurosurgery
University of Bern
Bern, Switzerland

G. A. Roberts
University of Bristol
Royal Preston Hospital
Bristol, UK

David S. Rosen, M.D.
Department of Surgery
Section of Neurosurgery
University of Chicago Medical Center
Pritzker School of Medicine
Chicago, IL

Stuart Ross, M.Ch., F.R.C.S. (S.N.)
Consultant Neurosurgeon
Department of Neurosurgery
Leeds General Infirmary
Leeds, UK

Devan Ryan, B.S.
Department of Neurosurgery
University of Chicago Medical Center
Chicago, IL

Müge Sahinbas, M.D.
Resident
Istanbul University
Istanbul Medical School
Istanbul, Turkey

Atsushi Saito, M.D.
Tohoku University School of Medicine
Tohoku, Japan

Nobuhito Saito, M.D., Ph.D.
Professor
Department of Neurosurgery
Gunma University School of Medicine
Gunma, Japan

Jun Sakuma, M.D.
Instructor
Department of Neurosurgery
Fukushima Medical University
Fukushima, Japan

Takahiro Sakuma, M.D.
Department of Neurosurgery
Fukui Medical University
Fukui, Japan

Hirotsugu Samejima, M.D.
Professor
Department of Neurosurgery
Toho University School of Medicine
Tokyo, Japan

Atsushi Sasahara, M.D.
Department of Neurosurgery
Tokyo Women's Medical University
Tokyo, Japan

Tatsuya Sasaki, M.D.
Associate Professor
Department of Neurosurgery
Fukushima Medical University
Fukushima, Japan

Tomio Sasaki, M.D., Ph.D.
Professor
Department of Neurosurgery
Neurological Institute
Graduate School of Medical Sciences
Kyushu University
Fukuoka, Japan

Toshiyuki Sasaki, M.D.
Senior Lecturer
Department of Neurosurgery
Tokyo Women's Medical University
Tokyo, Japan

Sonomi Sato, M.D.
Instructor
Department of Neurosurgery
Fukushima Medical University
Fukushima, Japan

Taku Sato, M.D.
Department of Neurosurgery
Fukushima Medical University
Fukushima, Japan

Shin-ichi Satoh
Department of Neurosurgery
Nagoya University School of Medicine
Nagoya, Japan

Claudia Schäfer, M.D.
Department of Physiology
University of Giessen
Giessen, Germany

Wolfram Scharbrodt, M.D.
Neurochirurgische Klinik des
Universitätsklinikums Gieben
Klinikstrabe, Germany

Lothar Schilling, Ph.D.
Faculty for Clinical Medicine
University of Heidelberg Mannheim
Mannheim, Germany

Richard H. Schmidt, M.D., Ph.D.
Associate Professor
Department of Neurosurgery
University of Utah
Salt Lake City, UT

Peter Schmiedek, M.D.
Ruprecht Karls Universitat Heidelberg
Heidelberg, Germany

Gerrit A. Schubert, M.D.
Department of Neurosurgery
University Hospital Mannheim
University of Heidelberg
Mannheim, Germany

Ute Schuhmacher, M.S.
Department of Neurosurgery
University Hospital Giessen
Giessen, Germany

Volker Seifert, M.D.
Department of Neurosurgery
Johann Wolfgang Goethe University
Frankfurt, Germany

Rolf W. Seller, M.D.
Chairman and Professor
Department of Neurosurgery
Inselspital Bern
University of Bern
Bern, Switzerland

Nirav Shah, M.D.
Department of Neurosurgery
University of Maryland School of Medicine
Baltimore, MD

Anu Sharma, M.B.B.S., D.A., F.F.A.R.C.S.I.
Department of Anaesthesia
Bradford Royal Infirmary
Bradford, UK

Frank R. Sharp, M.D., Ph.D.
MIND Institute
University of California–Davis
Sacramento, CA

Masato Shibuya, M.D.
Director
Chukyo Hospital
Nagoya, Japan

Minoru Shigemori, M.D.
Professor and Chairman
Department of Neurosurgery
Kurume University School of Medicine
Kurume, Japan

Hiroshi Shima, M.D.
Departments of Neurosurgery
Kanazawa University School of Medicine
Kanazawa, Japan

Tatsuya Shimizu, M.D.
Department of Neurosurgery
Gunma University School of Medicine
Gunma, Japan

J. Marc Simard, M.D., Ph.D.
Professor
University of Maryland
School of Medicine
Department of Neurosurgery
Baltimore, MD

Piotr Smielewski, Ph.D.
Wolfson Brain Imaging Centre and Department of Neurosurgery
Addenbrooke's Hospital
Cambridge, UK

Kenji Sugiu, M.D.
Department of Neurological Surgery
Okayama University Graduate School of Medicine and Dentistry
Okayama City, Japan

SuLun Sun, M.D.
Department of Neurology
DongZhiMen Hospital
Beijing University of Chinese Medicine
Beijing, China

Hidenori Suzuki, M.D., Ph.D.
Assistant Professor
Department of Neurosurgery
Mie University School of Medicine
Mie, Japan

Kyouichi Suzuki, M.D.
Assistant Professor
Department of Neurosurgery
Fukushima Medical University
Fukushima, Japan

Shigeharu Suzuki, M.D.
Professor Emeritus
Hirosaki University School of Medicine
Aomori City Hospital
Aomori, Japan

Yoshio Suzuki, M.D.
Department of Neurosurgery
Nagoya Daini Red Cross Hospital
Nagoya, Japan

Niels-Aage Svendgaard, M.D., Ph.D.
Professor Emeritus
Department of Clinical Experimental Research
Glostrup University Hospital
Glostrup, Denmark

Gill E. Sviri, M.D., M.Sc.
Instructor
Department of Neurological Surgery
University of Washington
Harborview Medical Center
Seattle, WA

Yoshihiro Takanashi, M.D., Ph.D.
Division of Neurosurgery
Yokohama Stroke and Brain Center
Yokohama, Japan

Yasuharu Takeuchi, M.D.
Staff Neurosurgeon
Department of Neurosurgery
Kurume University School of Medicine
Kurume, Japan

Waro Taki, M.D., Ph.D.
Professor
Mie University School of Medicine
Mie, Japan

Katsuhiro Tanaka, M.D.
Department of Neurosurgery
Ise Municipal Hospital
Mie, Japan

Alan Tennant, Ph.D.
Professor
Department of Medicine
University of Leeds
Leeds, UK

Susumu Terakawa, M.D., Ph.D.
Hamamatsu University School of Medicine
Laboratory of Cell Imaging
Photon Medical Research Center
Shizuoka, Japan

Claudius F. C. Thomé, M.D.
Department of Neurosurgery
University Hospital Mannheim
University of Heidelberg
Mannheim, Germany

Simon Thomson, M.B.B.S., F.R.C.S. (S.N.)
Specialist Registrar in Neurosurgery
Department of Neurosurgery
Leeds General Infirmary
Leeds, UK

Kouji Tokunaga, M.D.
Department of Neurological Surgery
Okayama University Medical School
Okayama, Japan

Takashi Tokutomi, M.D., Ph.D.
Professor
Department of Neurosurgery
Kurume University School of Medicine
Kurume, Japan

Masahiko Tosaka, M.D., Ph.D.
Assistant Professor
Department of Neurosurgery
Gunma University Graduate School of Medicine
Gunma, Japan

Ming-Yuan Tseng, M.D.
Academic Neurosurgery and Wolfson Brain Imaging Centre
University of Cambridge
Cambridge, UK

Akira Tsuchida, M.D.
Department of Neurosurgery
Fukui Medical University
Fukui, Japan

Donnie R. Tyler II, M.D.
Department of Neurosurgery
University of Mississippi Medical Center
Jackson, MS

John D. Tyler, B.S.
Department of Neurosurgery
University of Mississippi Medical Center
Jackson, MS

Morikazu Ueda, M.D.
Professor
Department of Neurosurgery
Toho University School of Medicine
Tokyo, Japan

Yosifumi Ueda, M.D.
Fukui, Japan

Hisao Uehara, M.D., Ph.D.
Assistant Professor
Department of Neurosurgery
Miyazaki Medical College
Miyazaki, Japan

Junichi Umekawa, M.D.
Division of Neurosurgery
Yokohama Stroke and Brain Center
Yokohama, Japan

Peter Vajkoczy, M.D.
Department of Neurosurgery
University of Heidelberg
Mannheim, Germany

Hartmut Vatter, M.D.
Department of Neurosurgery
Johann Wolfgang Goethe University
Frankfurt, Germany

Ramanathan Venkatachalam, F.R.C.S.
Lancashire and Division of Child Health (R.H.L.)
Royal Preston Hospital
University of Bristol
Bristol, UK

Andrea Vignati, M.D.
Assistant Professor
Institute of Neurosurgery
Catholic University School of Medicine
Rome, Italy

Shinichiro Wakisaka, M.D., Ph.D.
Professor
Department of Neurosurgery
Miyazaki Medical College
Miyazaki, Japan

George Weyer
Department of Neurosurgery
University of Chicago Medical Center
Chicago, IL

Roland Wiest, M.D.
Oberarzt Neurochirurgie
Inselspital Bern
Universität Bern
Bern, Switzerland

Cang-Bao Xu, M.D., Ph.D.
Senior Research Fellow
Department of Internal Medicine
Lund University Hospital
Lund, Sweden

Mitsuo Yamaguchi, M.D.
Louisiana State University Health Sciences Center
Shreveport, LA

Isao Yamamoto, M.D.
Department of Neurosurgery
Yokohama City University
Yokohama, Japan

Seiji Yamamoto, M.D., Ph.D.
Laboratory of Cell Imaging
Photon Medical Research Center
Hamamatsu University School of Medicine
Shizuoka, Japan

Junkoh Yamashita, M.D., Ph.D.
Department of Neurosurgery
Kanazawa University School of Medicine
Kanazawa, Japan

Masashi Yanagisawa, Ph.D.
University of Texas Southwestern Medical Center at Dallas
Dallas, TX

Reza Yassari, M.D.
Section of Neurosurgery
University of Chicago Medical Center
Chicago, IL

Tetsuya Yokouchi, M.D.
Department of Neurosurgery
Toho University School of Medicine
Tokyo, Japan

Taku Yoneyama, M.D.
Department of Neurosurgery
Tokyo Women's Medical University
Tokyo, Japan

Toshiyuki Yoshida, M.D.
Division of Neurosurgery
Yokohama Stroke and Brain Center
Yokohama, Japan

John H. Zhang, M.D., Ph.D.
Professor
Department of Neurosurgery
University of Mississippi Medical Center
Jackson, MS

Zhuang Zhang, Ph.D.
Department of Neurology
DongZhiMen Hospital
Beijing University of Chinese Medicine
Beijing, China

LiQun Zhong, M.D.
Department of Neurology
DongZhiMen Hospital
Beijing University of Chinese Medicine
Beijing, China

Mingfang Zhou, M.D.
Research Assistant
Department of Internal Medicine
Lund University Hospital
Lund, Sweden

Michael Zimmermann, M.D., Ph.D.
Department of Neurosurgery
Johann Wolfgang Goethe University
Frankfurt, Germany

Mario Zuccarello, M.D.
Professor
Vice Chair for Clinical Affairs
Department of Neurosurgery
University of Cincinnati College of Medicine
Cincinnati, OH

SECTION I

Molecular Mechanisms of Vasospasm

1

Signaling Pathways in Cerebral Vasospasm

JOHN H. ZHANG, M.D., PH.D.

A Medline search (keywords *vasospasm* and *cerebral)* for articles related to cerebral vasospasm published from 2000 to March 2003 selected more than 500 papers, some of which studied signaling pathways. Among them 15 papers studied mitogen-activated protein (MAP) kinases,[1–8] 50 papers studied protein kinase C,[9–3] 17 investigated tyrosine kinase,[9,10,14–16] and 15 examined Rho and Rho kinase.[10,12,13,17–21] Most of these papers covered more than one subject. In the interest of thoroughness, some papers on signaling pathways in cerebral vasospasm published before 2000 are also included in this brief review. Papers related to endothelin, nitric oxide, free radicals, and gene therapy are not included in this review.

The pathogenesis of cerebral vasospasm involves multiple signaling pathways involved in proliferation, inflammation, cell death, smooth muscle phenotype changes, vascular remodeling, and contraction.[22-24] A review of all of these areas is beyond the scope of this article, and as such, two systems that mediate these vascular responses have been selected: the tyrosine kinase–MAP kinase pathway and the sphingosine-1–Rho myosin light chain kinase pathway. Other signaling pathways, including protein kinase C, have been reviewed elsewhere.[25]

Tyrosine Kinase and Mitogen-Activated Protein Kinase

MAP kinase has been suggested to be one of the most important signaling pathways involved in cerebral vasospasm[25,26] based in part on the role of MAP kinase in cell differentiation, proliferation, contraction, death, and remodeling.[27,28] The role of MAP kinase in cerebral vasospasm may be summarized as follows:

1. MAP kinase is involved in the regulation of Ca^{2}+ in cerebral smooth muscle cells[29] and in the contraction of cerebral arteries.[4,6–8,30–32]
2. Blood clot components including hemoglobin and vasoactive agents released from the vessel wall such as endothelin-1 enhance MAP kinase expression and activity in cerebral arteries.[4,6–8,30] Free radicals activate MAP kinase in vascular smooth muscle cells.[33]
3. MAP kinase inhibitors were reported to decrease MAP kinase expression in vasospastic arteries and to reduce the degree of cerebral vasospasm in animal models.[1,3,31,32] A recent study demonstrated that antisense oligodeoxynucleotides to MAP kinase abolished MAP kinase activity and phosphorylated MAP kinase and reduced vasospasm in a rat model of subarachnoid hemorrhage.[2]

Because MAP kinase is a substrate of tyrosine kinase, the possible role of tyrosine kinase as an upstream modulator of MAP kinase in cerebral vasospasm needs to be addressed. The mechanisms of tyrosine kinase–induced contraction in smooth muscle have been summarized.[34]

1. Tyrosine kinase regulates intracellular Ca^{2+} ($[Ca^{2+}]_i$) and smooth muscle contraction induced by spasmogens including blood clot components and vasoactive agents.[35–38] Tyrosine kinase is involved in the compaction of fibroblasts.[16,39]
2. Some G-protein coupled receptor agonists and growth factors activate tyrosine kinase. The level of these G-protein receptor agonists such as adenosine triphosphate and endothelins,[40–49] growth factors,[50-55] and their receptors are increased in cerebrospinal fluid or in cerebral arteries.
3. Tyrosine activation phosphorylates other substrates such as Ras protein and phosphatidyl inositol-3 kinase tyrosine kinase. Ras is increased after spasmogen stimulation,[16] and phosphatidylinositol-3 kinase is enhanced after vasospasm in animals.[15]

4. The tyrosine kinase inhibitor suramin but not phosphatidylinositol-3 kinase inhibitors reduced vasospasm in animal models.[14,15]

There are some interactions between MAP kinases and tyrosine kinases. For example, oxidative stress activates extracellular signal-regulated kinases through Src and Ras in cultured cardiac myocytes of neonatal rats.[56] Coupling of c-Src to large conductance voltage- and Ca^{2+}-activated K+ channels was reported as a new mechanism of agonist-induced vasoconstriction.[57] Src is a major cytosolic tyrosine kinase in vascular tissue.[58] Angiotensin II controls p21ras activity via pp60c-src.[59] Src tyrosine kinase and phosphatidylinositol-3 kinase are upstream of MAP kinase.[60–62] Hemoglobin enhances the expression of Src in cerebral smooth muscle cells.[4] Src inhibitors attenuated cerebral vasospasm in animal models (J. Zhang, unpublished observations). When compared with protein kinase C, it was reported that the contractile property of cerebral arteries shifted from active myogenic tone involving protein kinase C to nonmyogenic tone involving tyrosine kinases to produce prolonged vasospasm in a dog model.[9]

Overall, MAP kinase and particularly the extracellular regulated kinase (ERK) system is involved in proliferation, differentiation, and contraction of cerebral arteries after subarachnoid hemorrhage. Other MAP kinases such as p38 may be involved in stress-induced cell death, especially apoptosis,[63–68] that has been investigated but is not discussed in this article.

Sphingosine-1 and Rho/Myosin Light Chain Kinase

Sphingosine 1-phosphate (S1P) is a polar lysophospholipid metabolite that is stored in platelets and released on their activation. Similar to MAP kinase, diverse stimuli such as growth factors, cytokines, G-protein coupled receptor agonists, and antigens have been shown to increase sphingosine kinase activity and S1P formation in different cell types, including smooth muscle cells. Indeed, S1P has been implicated in the regulation of several important cellular processes, such as proliferation, differentiation, apoptosis, and migration in these cells.[69]

Sphingosylphosphorylcholine (SPC), a sphingolipid, is a novel messenger for Rho kinase-mediated Ca^{2+} sensitization in the bovine cerebral artery but not for protein kinase C.[12] Sphingosylphosphorylcholine induced translocation of cytosolic Rho kinase to the cell membrane and produced contraction of bovine middle cerebral artery without increasing $[Ca^{2+}]_i$. Rho kinase inhibitor Y-27632 and a dominant-negative Rho kinase blocked SPC-induced Ca^{2+} sensitization.[12]

Rho A and Rho kinase are believed to play roles in smooth muscle contraction and cytoskeleton reorganization.[70,71] Adenovirus-mediated transfer of dominant-negative Rho kinase induces a regression of coronary arteriosclerosis in pigs in vivo,[72] and Rho mediates contraction of rabbit basilar artery to endothelin-1.[73] The mechanisms may relate to myosin light chain kinase because hydroxyfasudil, an active metabolite of fasudil hydrochloride, relaxes the rabbit basilar artery by disinhibition of myosin light chain phosphatase.[18] Rho kinase, which is activated by the small guanosine triphosphatase Rho, phosphorylates not only myosin light chain but also myosin phosphatase at its myosin-binding subunit, thus inactivating myosin phosphatase. Activation of Rho kinase and the phosphorylation of myosin light chain and myosin-binding subunit occur concomitantly during vasospasm and enhance myosin light chain kinase by increasing the phosphorylation of myosin light chain directly or indirectly because of the inhibition of myosin phosphatase by its phosphorylation.[20]

The possible roles of S1P and Rho in cerebral vasospasm are summarized in the following:

1. S1P contracts canine basilar arteries in vitro and in vivo by activation of Rho/Rho kinase.[17] Rho kinase inhibitor Y-27632 abolished the effect of S1P.[17] Rho kinase inhibitors Y-27632 and HA-1077 reduced sustained contraction induced by oxyhemoglobin.[13] Oxyhemoglobin produced Rho translocation, which was inhibited by GGTI-297, an inhibitor of Rho prenylation. Translocation of protein kinase C a and protein kinase C e was observed in the same tissues.[13]
2. The level of S1P in the supernatant of clot increased.[17] Administration of S1P into cerebrospinal fluid produced cerebral vasospasm that lasts for 2 days.[17]
3. Rho A and Rho kinase expression are enhanced in cerebral arteries during vasospasm.[19] The Rho kinase activation levels in vasospasm are comparable to those in KCl- and serotorin-induced vasoconstriction.[74]
4. A selective inhibitor of Rho kinase, Y-17632, abolished vasospasm in a dog model.[20] A nonselective Rho kinase inhibitor or protein kinase inhibitor, fasudil, reduced vasospasm in an animal model of vasospasm[75] by disinhibition of myosin light chain phosphatase.[18] Several clinical studies have been published regarding the therapeutic effect of fasudil, especially in Japan.[76–79]

Protein kinase C has been reviewed and will not be further discussed.[25-80] A recent study indicates protein kinase C may be involved in the initial contraction but

not the late-stage contraction (vasospasm) in animal models.[11] Degradation of the thin filament-associated protein calponin in spastic arteries in an animal model has been reported.[81] HA1077, an inhibitor of protein kinases, including Rho kinase and myosin light chain kinase, reduced vasospasm and calponin degradation in this model.

Other signaling pathways that may be involved in vasospasm include those activated by or involved in the contractions of cerebral arteries related to endothelin,[82] nitric oxide synthase,[83] bilirubin,[84] matrix metalloproteinases,[55] adhesion molecules,[85] protein C,[86] cyclic adenosine monophosphate/phosphodiesterase,[87] cyclic guanosine monophosphate,[88] parathyroid hormone,[22] erythropoietin,[89–91] and nicotinamide adenine dinucleotide phosphate oxidase.[92] Additional research is required to clarify the roles of these pathways and agents in vasospasm.

Acknowledgment

This work was partially supported by grants from the American Heart Association Bugher Foundation Award for Stroke Research and the National Institutes of Health (NS45694).

REFERENCES

1. Aoki K, Zubkov AY, Tibbs RE, Zhang JH. Role of MAPK in chronic cerebral vasospasm. Life Sci 2002;70:1901–1908
2. Satoh M, Parent AD, Zhang JH. Inhibitory effect with antisense mitogen-activated protein kinase oligodeoxynucleotide against cerebral vasospasm in rats. Stroke 2002;33:775–781
3. Tibbs R, Zubkov A, Aoki K, et al. Effects of mitogen-activated protein kinase inhibitors on cerebral vasospasm in a double-hemorrhage model in dogs. J Neurosurg 2000;93:1041–1047
4. Vollrath B, Cook D, Megyesi J, Findlay JM, Ohkuma H. Novel mechanism by which hemoglobin induces constriction of cerebral arteries. Eur J Pharmacol 1998;361:311–319
5. Yin W, Tibbs R, Aoki K, Badr A, Zhang J. Metabolic alterations in cerebrospinal fluid from double hemorrhage model of dogs. Acta Neurochir Suppl 2002;81:257–263
6. Zubkov AY, Ogihara K, Tumu P, et al. Mitogen-activated protein kinase mediation of hemolysate-induced contraction in rabbit basilar artery. J Neurosurg 1999;90:1091–1097
7. Zubkov AY, Rollins KS, McGehee B, Parent AD, Zhang JH. Relaxant effect of U0126 in hemolysate-, oxyhemoglobin-, and bloody cerebrospinal fluid-induced contraction in rabbit basilar artery. Stroke 2001;32:154–161
8. Zubkov AY, Rollins KS, Parent AD, Zhang J, Bryan JM Jr. Mechanism of endothelin-1-induced contraction in rabbit basilar artery. Stroke 2000;31:526–533
9. Koide M, Nishizawa S, Ohta S, Yokoyama T, Namba H. Chronological changes of the contractile mechanism in prolonged vasospasm after subarachnoid hemorrhage: from protein kinase C to protein tyrosine kinase. Neurosurgery 2002;51:1468–1474
10. Nakayama K, Obara K, Tanabe Y, Saito M, Ishikawa T, Nishizawa S. Interactive role of tyrosine kinase, protein kinase C, and Rho/Rho kinase systems in the mechanotransduction of vascular smooth muscles. Biorheology 2003;40:307–314
11. Nishizawa S, Chen D, Yokoyama T, Yokota N, Ohta S. Endothelin-1 initiates the development of vasospasm after subarachnoid haemorrhage through protein kinase C activation, but does not contribute to prolonged vasospasm. Acta Neurochir Suppl 2000; 142:1409–1415
12. Shirao S, Kashiwagi S, Sato M, et al. Sphingosylphosphorylcholine is a novel messenger for Rho-kinase-mediated Ca^{2+} sensitization in the bovine cerebral artery: unimportant role for protein kinase C. Circ Res 2002;91:112–119
13. Wickman G, Lan C, Vollrath B. Functional roles of the rho/rho kinase pathway and protein kinase C in the regulation of cerebrovascular constriction mediated by hemoglobin: relevance to subarachnoid hemorrhage and vasospasm. Circ Res 2003; 92:809–816
14. Kimura H, Meguro T, Badr A, Zhang JH. Suramin-induced reversal of chronic cerebral vasospasm in experimental subarachnoid hemorrhage. J Neurosurg 2002;97:129–135
15. Kimura H, Sasaki K, Meguro T, Zhang JH. Phosphatidylinositol 3-kinase inhibitor failed to reduce cerebral vasospasm in dog model of experimental subarachnoid hemorrhage. Stroke 2002; 33:593–599
16. Patlolla A, Ogihara K, Zubkov A, Aoki K, Parent AD, Zhang JH. Role of tyrosine kinase in fibroblast compaction and cerebral vasospasm. Acta Neurochir Suppl 2000;76:227–230
17. Tosaka M, Okajima F, Hashiba Y, et al. Sphingosine 1-phosphate contracts canine basilar arteries in vitro and in vivo: possible role in pathogenesis of cerebral vasospasm. Stroke 2001;32: 2913–2919
18. Nakamura K, Nishimura J, Hirano K, Ibayashi S, Fujishima M, Kanaide H. Hydroxyfasudil, an active metabolite of fasudil hydrochloride, relaxes the rabbit basilar artery by disinhibition of myosin light chain phosphatase. J Cereb Blood Flow Metab 2001;21:876–885
19. Miyagi Y, Carpenter RC, Meguro T, Parent AD, Zhang JH. Upregulation of rho A and rho kinase messenger RNAs in the basilar artery of a rat model of subarachnoid hemorrhage. J Neurosurg 2000;93:471–476
20. Sato M, Tani E, Fujikawa H, Kaibuchi K. Involvement of Rho-kinase-mediated phosphorylation of myosin light chain in enhancement of cerebral vasospasm. Circ Res 2000;87:195–200
21. Sato M, Tani E, Fujikawa H, Yamaura I, Arita N, Kaibuchi K. Importance of Rho-kinase-mediated phosphorylation of myosin light chain in vasospasm. Acta Neurochir Suppl 2001;77:49–54
22. Macdonald RL, Zhang ZD, Ono S, Komuro T. Up-regulation of parathyroid hormone receptor in cerebral arteries after subarachnoid hemorrhage in monkeys. Neurosurgery 2002;50: 1083–1091
23. Smith RR, Clower BR, Grotendorst GM, Yabuno N, Cruse JM. Arterial wall changes in early human vasospasm. Neurosurgery 1985;16:171–176
24. Zhang J, Lewis A, Bernanke D, Zubkov A, Clower B. Stroke: anatomy of a catastrophic event. Anat Rec 1998;253:58–63
25. Laher I, Zhang JH. Protein kinase C and cerebral vasospasm. J Cereb Blood Flow Metab 2001;21:887–906
26. Asano T. Oxyhemoglobin as the principal cause of cerebral vasospasm: a holistic view of its actions. Crit Rev Neurosurg 1999;9:303–318
27. Page K, Hershenson MB. Mitogen-activated signaling and cell cycle regulation in airway smooth muscle. Front Biosci 2000;5: D258–D267
28. Zhu W, Zou Y, Aikawa R, et al. MAPK superfamily plays an important role in daunomycin-induced apoptosis of cardiac myocytes. Circulation 1999;100:2100–2107
29. Aoki K, Williams R, Zhang JH. Mechanism of hemolysate-induced $[Ca^{2+}]_i$ elevation in cultured fibroblasts. Neurol Res 2001;23:367–373
30. Zubkov AY, Ogihara K, Patllola A, Parent AD, Zhang J. Mitogen-activated protein kinase plays an important role in hemolysate-induced contraction in rabbit basilar artery. Acta Neurochir Suppl 2000;76:217–221
31. Zubkov AY, Tibbs RE, Aoki K, Zhang JH. Prevention of vasospasm in penetrating arteries with MAPK inhibitors in dog double-hemorrhage model. Surg Neurol 2000;54:221–227
32. Fujikawa H, Tani E, Yamaura I, et al. Activation of protein kinases in canine basilar artery in vasospasm. J Cereb Blood Flow Metab 1999;19:44–52

33. Baas AS, Berk BC. Differential activation of mitogen-activated protein kinases by H_2O_2 and O_2 - in vascular smooth muscle cells. Circ Res 1995;77:29–36

34. Hughes AD, Wijetunge S. Role of tyrosine phosphorylation in excitation–contraction coupling in vascular smooth muscle. Acta Physiol Scand 1998;164:457–469

35. Iwabuchi S, Marton LS, Zhang JH. Role of protein tyrosine phosphorylation in erythrocyte lysate–induced intracellular free calcium concentration elevation in cerebral smooth-muscle cells. J Neurosurg 1999;90:743–751

36. Marton LS, Weir BK, Zhang H. Tyrosine phosphorylation and $[Ca^{2+}]_i$ elevation induced by hemolysate in bovine endothelial cells: implications for cerebral vasospasm. Neurol Res 1996;18: 349–353

37. Kim CJ, Kim KW, Park JW, Lee JC, Zhang JH. Role of tyrosine kinase in erythrocyte lysate–induced contraction in rabbit cerebral arteries. J Neurosurg 1998;89:289–296

38. Watanabe M, Doi M, Sasaki K, Ogawa A. Modulatory role of protein tyrosine kinase activation in the receptor-induced contractions of the bovine cerebral artery. Neurol Med Chir (Tokyo) 1998;38:75–81

39. Patlolla A, Zubkov A, Parent A, Zhang J. Hemolysate activates P21RAS in rabbit basilar artery. Life Sci 2000;67:1233–1242

40. Erlinge D. Extracellular ATP: a growth factor for vascular smooth muscle cells. Gen Pharmacol 1998;31:1–8

41. Yin W, Tibbs R, Tang J, Badr A, Zhang J. Haemoglobin and ATP levels in CSF from a dog model of vasospasm. J Clin Neurosci 2002;9:425–428

42. Kim CJ, Weir BK, Macdonald RL, Zhang H. Erythrocyte lysate releases Ca^{2+} from IP_3-sensitive stores and activates Ca^{2+}-dependent K^+ channels in rat basilar smooth muscle cells. Neurol Res 1998;20:23–30

43. Sima B, Weir BK, Macdonald RL, Zhang H. Extracellular nucleotide-induced $[Ca^{2+}]_i$ elevation in rat basilar smooth muscle cells. Stroke 1997;28:2053–2058

44. Zhang H, Weir BK, Macdonald RL, et al. Mechanisms of $[Ca^{++}]_i$ elevation induced by erythrocyte components in endothelial cells. J Pharmacol Exp Ther 1996;277:1501–1509

45. Zubkov AY, Ogihara K, Tumu P, et al. Bloody cerebrospinal fluid alters contractility of cultured arteries. Neurol Res 1999;21: 553–558

46. Asano T, Ikegaki I, Satoh S, et al. Endothelin: a potential modulator of cerebral vasospasm. Eur J Pharmacol 1990;190:365–372

47. Clozel M. Endothelin receptor antagonists: current status and perspectives. J Cardiovasc Pharmacol 2000;35:S65–S68

48. Fassbender K, Hodapp B, Rossol S, et al. Endothelin-1 in subarachnoid hemorrhage: an acute-phase reactant produced by cerebrospinal fluid leukocytes. Stroke 2000;31:2971–2975

49. Jeng AY, Mulder P, Kwan AL, Battistini B. Nonpeptidic endothelin-converting enzyme inhibitors and their potential therapeutic applications. Can J Physiol Pharmacol 2002;80:440–449

50. Aihara Y, Kasuya H, Onda H, Hori T, Takeda J. Quantitative analysis of gene expressions related to inflammation in canine spastic artery after subarachnoid hemorrhage. Stroke 2001;32: 212–217

51. Cuevas P, Carceller F, Nieto I, Gimenez-Gallego G. Spasmolytic effect of acidic fibroblast growth factor in early cerebral vasospasm in the rat. Surg Neurol 1998;49:176–179

52. Ogane K, Wolf EW, Robertson JH. Role of basic fibroblast growth factor in the course of cerebral vasospasm in an experimental model of subarachnoid hemorrhage. Neurol Res 2002; 24:365–372

53. Vieweg U, Schramm J, Urbach H. Platelet-derived growth factor (PDGF-AB) like immune reactivity in serum and in cerebral spinal fluid following experimental subarachnoid haemorrhage in dogs. Acta Neurochir (Wien) 1999;141:861–865

54. Zhang Z, Nagata I, Kikuchi H, et al. Broad-spectrum and selective serine protease inhibitors prevent expression of platelet-derived growth factor-BB and cerebral vasospasm after subarachnoid hemorrhage: vasospasm caused by cisternal injection of recombinant platelet-derived growth factor-BB. Stroke 2001;32:1665–1672

55. McGirt MJ, Lynch JR, Blessing R, Warner DS, Friedman AH, Laskowitz DT. Serum von Willebrand factor, matrix metalloproteinase-9, and vascular endothelial growth factor levels predict the onset of cerebral vasospasm after aneurysmal subarachnoid hemorrhage. Neurosurgery 2002;51:1128–1134

56. Aikawa R, Komuro I, Yamazaki T, et al. Oxidative stress activates extracellular signal-regulated kinases through Src and Ras in cultured cardiac myocytes of neonatal rats. J Clin Invest 1997;100:1813–1821

57. Alioua A, Mahajan A, Nishimaru K, Zarei MM, Stefani E, Toro L. Coupling of c-Src to large conductance voltage- and Ca^{2+}-activated K+ channels as a new mechanism of agonist-induced vasoconstriction. Proc Natl Acad Sci USA 2002;99:14560–14565

58. Oda Y, Renaux B, Bjorge J, Saifeddine M, Fujita DJ, Hollenberg MD. cSrc is a major cytosolic tyrosine kinase in vascular tissue. Can J Physiol Pharmacol 1999;77:606–617

59. Schieffer B, Paxton WG, Chai Q, Marrero MB, Bernstein KE. Angiotensin II controls p21ras activity via pp60c-src. J Biol Chem 1996;271:10329–10333

60. Dikic I, Tokiwa G, Lev S, Courtneidge SA, Schlessinger J. A role for Pyk2 and Src in linking G-protein-coupled receptors with MAP kinase activation. Nature 1996;383:547–550

61. Hawes BE, Luttrell LM, van Biesen T, Lefkowitz RJ. Phosphatidylinositol 3-kinase is an early intermediate in the G beta gamma–mediated mitogen-activated protein kinase signaling pathway. J Biol Chem 1996;271:12133–12136

62. Luttrell LM, Hawes BE, van Biesen T, Luttrell DK, Lansing TJ, Lefkowitz RJ. Role of c-Src tyrosine kinase in G protein-coupled receptor- and G beta gamma subunit-mediated activation of mitogen-activated protein kinases. J Biol Chem 1996;271:19443–19450

63. Akin E, Clower B, Tibbs R, Tang J, Zhang J. Bilirubin produces apoptosis in cultured bovine brain endothelial cells. Brain Res 2002;931:168–175

64. Meguro T, Klett CP, Chen B, Parent AD, Zhang JH. Role of calcium channels in oxyhemoglobin-induced apoptosis in endothelial cells. J Neurosurg 2000;93:640–646

65. Meguro T, Chen B, Parent AD, Zhang JH. Caspase inhibitors attenuate oxyhemoglobin-induced apoptosis in endothelial cells. Stroke 2001;32:561–566

66. Meguro T, Chen B, Lancon J, Zhang JH. Oxyhemoglobin induces caspase-mediated cell death in cerebral endothelial cells. J Neurochem 2001;77:1128–1135

67. Ogihara K, Zubkov AY, Bernanke DH, Lewis AI, Parent AD, Zhang JH. Oxyhemoglobin-induced apoptosis in cultured endothelial cells. J Neurosurg 1999;91:459–465

68. Ogihara K, Zubkov AY, Parent AD, Zhang JH. Oxyhemoglobin produces necrosis in cultured smooth muscle cells. Acta Neurochir Suppl 2000;76:507–510

69. Pyne S, Pyne NJ. Sphingosine 1-phosphate signaling and termination at lipid phosphate receptors. Biochim Biophys Acta 2002;1582:121–131

70. Fukata Y, Amano M, Kaibuchi K. Rho-Rho-kinase pathway in smooth muscle contraction and cytoskeletal reorganization of non-muscle cells. Trends Pharmacol Sci 2001;22:32–39

71. Kawano Y, Yoshimura T, Kaibuchi K. Smooth muscle contraction by small GTPase Rho. Nagoya J Med Sci 2002;65:1–8

72. Morishige K, Shimokawa H, Eto Y, et al. Adenovirus-mediated transfer of dominant-negative rho-kinase induces a regression of coronary arteriosclerosis in pigs in vivo. Arterioscler Thromb Vasc Biol 2001;21:548–554

73. Miao L, Dai Y, Zhang J. Mechanism of RhoA/Rho kinase activation in endothelin-1-induced contraction in rabbit basilar artery. Am J Physiol Heart Circ Physiol 2002;283:H983–H989

74. Sato M, Tani E, Fujikawa H, et al. Importance of Rho-kinase-mediated phosphorylation of myosin light chain in cerebral vasospasm. J Stroke Cerebrovasc Dis 2000;9(suppl 1):223–224

75. Satoh S, Yamamoto Y, Toshima Y, et al. Fasudil, a protein kinase inhibitor, prevents the development of endothelial injury and neutrophil infiltration in a two-haemorrhage canine subarachnoid model. J Clin Neurosci 1999;6:394–399

76. Masaoka H, Takasato Y, Nojiri T, et al. Clinical effect of fasudil hydrochloride for cerebral vasospasm following subarachnoid hemorrhage. Acta Neurochir Suppl 2001;77:209–211

77. Nakashima S, Tabuchi K, Shimokawa S, Fukuyama K, Mineta T, Abe M. Combination therapy of fasudil hydrochloride and ozagrel sodium for cerebral vasospasm following aneurysmal subarachnoid hemorrhage. Neurol Med Chir (Tokyo) 1998;38:805–810
78. Shibuya M, Asano T, Sasaki Y. Effect of fasudil HCl, a protein kinase inhibitor, on cerebral vasospasm. Acta Neurochir Suppl 2001;77:201–204
79. Tachibana E, Harada T, Shibuya M, et al. Intra-arterial infusion of fasudil hydrochloride for treating vasospasm following subarachnoid haemorrhage. Acta Neurochir (Wien) 1999;141:13–19
80. Asano T, Matsui T. Various pathogenetic factors revolving around the central role of protein kinase C activation in the occurrence of cerebral vasospasm. Crit Rev Neurosurg 1998;8:176–187
81. Kim I, Leinweber BD, Morgalla M, et al. Thin and thick filament regulation of contractility in experimental cerebral vasospasm. Neurosurgery 2000;46:440–447
82. Zuccarello M. Endothelin: the "prime suspect" in cerebral vasospasm. Acta Neurochir Suppl 2001;77:61–65
83. Pluta RM, Afshar JK, Thompson BG, Boock RJ, Harvey-White J, Oldfield EH. Increased cerebral blood flow but no reversal or prevention of vasospasm in response to L-arginine infusion after subarachnoid hemorrhage. J Neurosurg 2000;92:121–126
84. Clark JF, Reilly M, Sharp FR. Oxidation of bilirubin produces compounds that cause prolonged vasospasm of rat cerebral vessels: a contributor to subarachnoid hemorrhage-induced vasospasm. J Cereb Blood Flow Metab 2002;22:472–478
85. Mocco J, Mack WJ, Kim GH, et al. Rise in serum soluble intercellular adhesion molecule-1 levels with vasospasm following aneurysmal subarachnoid hemorrhage. J Neurosurg 2002;97: 537–541
86. Mizuno Y, Azuma H, Ito Y, Isotani E, Ohno K, Hirakawa K. Inhibitory effect of activated protein C on cerebral vasospasm after subarachnoid hemorrhage in the rabbit. J Cardiovasc Pharmacol 2002;39:729–738
87. Nakamizo A, Inamura T, Inoha S, Amano T, Ikezaki K. Impaired intracellular signal transduction via cyclic AMP contributes to cerebral vasospasm in rats with subarachnoid hemorrhage. Neurol Res 2002;24:281–285
88. Inoha S, Inamura T, Ikezaki K, Nakamizo A, Amano T, Fukui M. Type V phosphodiesterase expression in cerebral arteries with vasospasm after subarachnoid hemorrhage in a canine model. Neurol Res 2002;24:607–612
89. Alafaci C, Salpietro F, Grasso G, et al. Effect of recombinant human erythropoietin on cerebral ischemia following experimental subarachnoid hemorrhage. Eur J Pharmacol 2000;406: 219–225
90. Grasso G, Buemi M, Alafaci C, et al. Beneficial effects of systemic administration of recombinant human erythropoietin in rabbits subjected to subarachnoid hemorrhage. Proc Natl Acad Sci USA 2002;99:5627–5631
91. Grasso G. Neuroprotective effect of recombinant human erythropoietin in experimental subarachnoid hemorrhage. J Neurosurg Sci 2001;45:7–14
92. Kim DE, Suh YS, Lee MS, et al. Vascular NAD(P)H oxidase triggers delayed cerebral vasospasm after subarachnoid hemorrhage in rats. Stroke 2002;33:2687–2691

2

A New Approach to the Mechanism of Smooth Muscle Contraction: Calmodulin Sensor MLC Kinase Mice

EIJI ISOTANI, M.D., PH.D.

Abstract

Ca^{2+}/calmodulin-dependent phosphorylation of myosin regulatory light chain by myosin light chain (MLC) kinase in smooth muscle is subject to modulatory cascades. We designed a calmodulin-sensor MLC kinase containing short MLC kinase fused to two fluorescent proteins, enhanced yellow and cyan fluorescent proteins that were linked by the calmodulin binding sequence. Ca^{2+}/calmodulin binding proportionally increased both MLC kinase activity and ratio of fluorescence emission at 480 to 525 nm (R480/525). A plasmid encoding calmodulin-sensor MLC kinase with the smooth muscle a-actin promoter was used to create transgenic mice. Immunoblotting and confocal microscopy showed calmodulin-sensor MLC kinase expressed specifically in smooth muscle tissues. Simultaneous measurements of R480/525 and force in skinned smooth muscle at pCa 6.0 plus 0.5 µmol/L calmodulin resulted in 28% and 8% of the respective maximal responses at pCa 3.7. The greater extent of MLC kinase activation at pCa 6.0 relative to force is probably necessary to exceed MLC phosphatase activity In KCl-stimulated intact smooth muscle the R480/525 increased to 53% of the maximal value measured after permeabilization. In agonist-induced contraction MLC kinase was activated only to 35%. Y27632, a specific Rho kinase inhibitor, abolished both KCl-induced contraction and MLC kinase activity. In agonist-stimulated smooth muscle the contraction was abolished, but MLC kinase activity was not reduced by Y2F7632. Calphostin C, a protein kinase C inhibitor, did not cause significant inhibition of either contraction or MLC kinase activity. Rho kinase has an important role in both KCl-induced (Ca^{2+}-dependent) and agonist-induced (Ca^{2+}-independent) contractile pathways. MLC kinase was not fully activated during depolarization-induced contraction. Smooth muscle tissue from these mice provides new insights into the modulation of MLC kinase activity in vivo.

The potential importance of myosin regulatory light chain phosphorylation in pathophysiological processes involving smooth muscle contraction is apparent, although the involvement of this process in cerebral vasospasm has been investigated less frequently. Ca^{2+}/calmodulin-dependent phosphorylation of the myosin regulatory light chain by MLC kinase in smooth muscle is subject to modulatory cascades.[1] New fluorescent technology has been developed that utilizes changes in fluorescence resonance energy transfer (FRET) between variants of green fluorescent protein. FRET is a distance-dependent interaction between two dye molecules where excitation of one molecule can be transferred to the second molecule

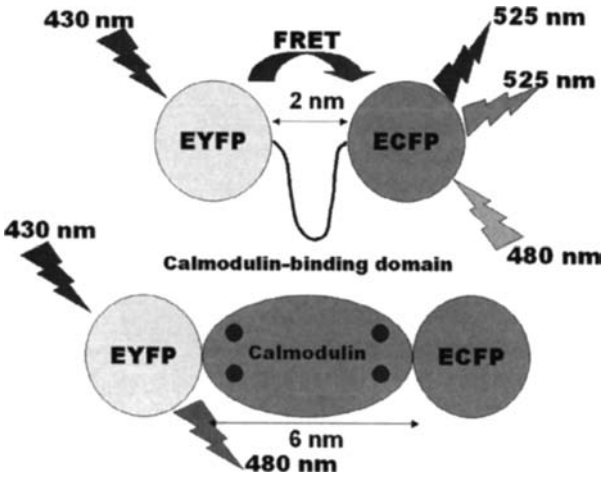


FIGURE 2–1 Diagram showing the principle of fluorescence resonance energy transfer (FRET). In the native state, enhanced yellow fluorescent protein (EYFP) and enhanced cyan fluorescent protein (ECFP) are linked by a peptide that can bind calmodulin. In the absence of calmodulin, excitation of EYFP at 430 nm leads to FRET to ECFP and emission of photons at 525 nm (top row). Binding of calmodulin, however, separates the EYFP and ECFP such that excitation of EYFP no longer can excite ECFP by FRET (bottom row).

without emission of a photon. The second molecule then emits a photon in some cases, the detection of which thus proves that the two dyes were in close proximity (typically 1 to 10 nm). The changes in FRET can be used to monitor cleavage at a protease site within a linker amino acid sequence.[2] We have designed a similar fluorescent indicator protein in which green fluorescent protein variants are linked by a calmodulin-binding sequence from MLC kinase. This indicator exhibits a large Ca^{2+}/calmodulin-dependent change in its fluorescence emission due to disruption of FRET when calmodulin is bound to the linker sequence (Fig. 2–1). This response can be monitored in living cells, where it closely follows changes in the intracellular Ca^{2+} ($[Ca^{2+}]_i$) concentration. The purposes of this study are to verify correlations between smooth muscle contraction, $[Ca^{2+}]_i$, and the concentration and activity of MLC kinase in intact smooth muscle tissues and to determine the role of Rho kinase and protein kinase C during smooth muscle contraction.

Methods

We designed a calmodulin-sensor MLC kinase containing short MLC kinase fused to two fluorescent proteins, enhanced yellow fluorescent protein and enhanced cyan fluorescent protein, linked by the calmodulin-binding sequence of MLC kinase. Ca^{2+}/calmodulin binding proportionally increased both MLC kinase activity and the ratio of fluorescence emission at 480 to 525 nm (R480/525, see Fig. 2–1). A plasmid encoding calmodulin-sensor MLC kinase with the smooth muscle a-actin promoter was used to create transgenic mice. Immunoblotting and confocal microscopy showed calmodulin-sensor MLC kinase was expressed specifically in smooth muscle tissues. Isolated smooth muscle tissue was attached to the transducer and mounted into the small quartz chamber incubated by aerated physiological saline solution. After equilibration, calmodulin sensor MLC kinase in smooth muscle tissue was excited by irradiation at 430 nm, and emission at 480 nm and 525 nm was detected by a Guth apparatus. The preparation was incubated in sequential bioactive solutions as follows: 65 mmol/L KCl in physiological saline solution, 100 mmol/L carbachol in physiological saline solution (for agonist stimulated smooth muscle), 4 mmol/L ethylene glycol-O, O'-bis (2-amino-ethyl)-tetraacetic acid (EGTA) Ca^{2+} free solution, ß-escin containing Ca^{2+} free solution (skinning solution), and a solution with a concentration of Ca^{2+} of 0.2 mmol/L (pCa 3.7 solution). The pCa is an expression of $[Ca^{2+}]$ similar to the convention for pH where pCa is the negative common log of the $[Ca^{2+}]$.

Results

Simultaneous measurements of R480/525 and force in muscle exposed to KCl, 65 mmol/L or to carbachol, 100 µmol/L shows that both produce phasic followed by tonic increases in force but that the FRET response differs (Fig. 2–2). Simultaneous measurements of

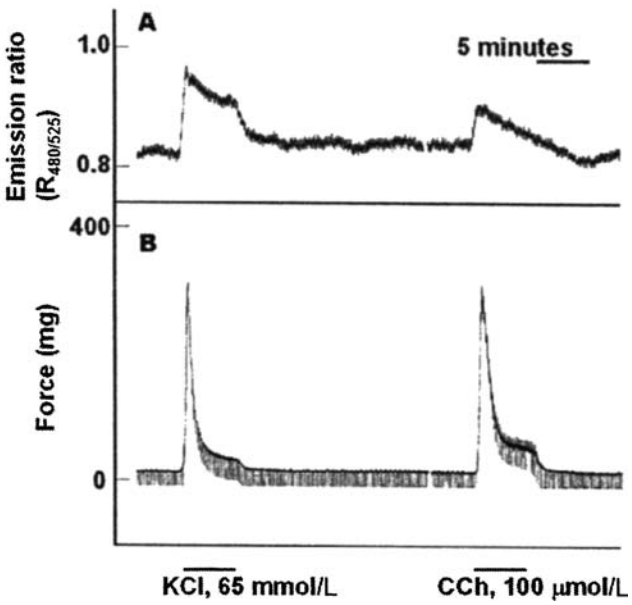


FIGURE 2–2 Simultaneous measurements of R480/525 and force in muscle exposed to KCl, 65 mmol/L or to carbachol (CCh), 100 µmol/L shows that both produce phasic followed by tonic increases in force but that the FRET response differs.

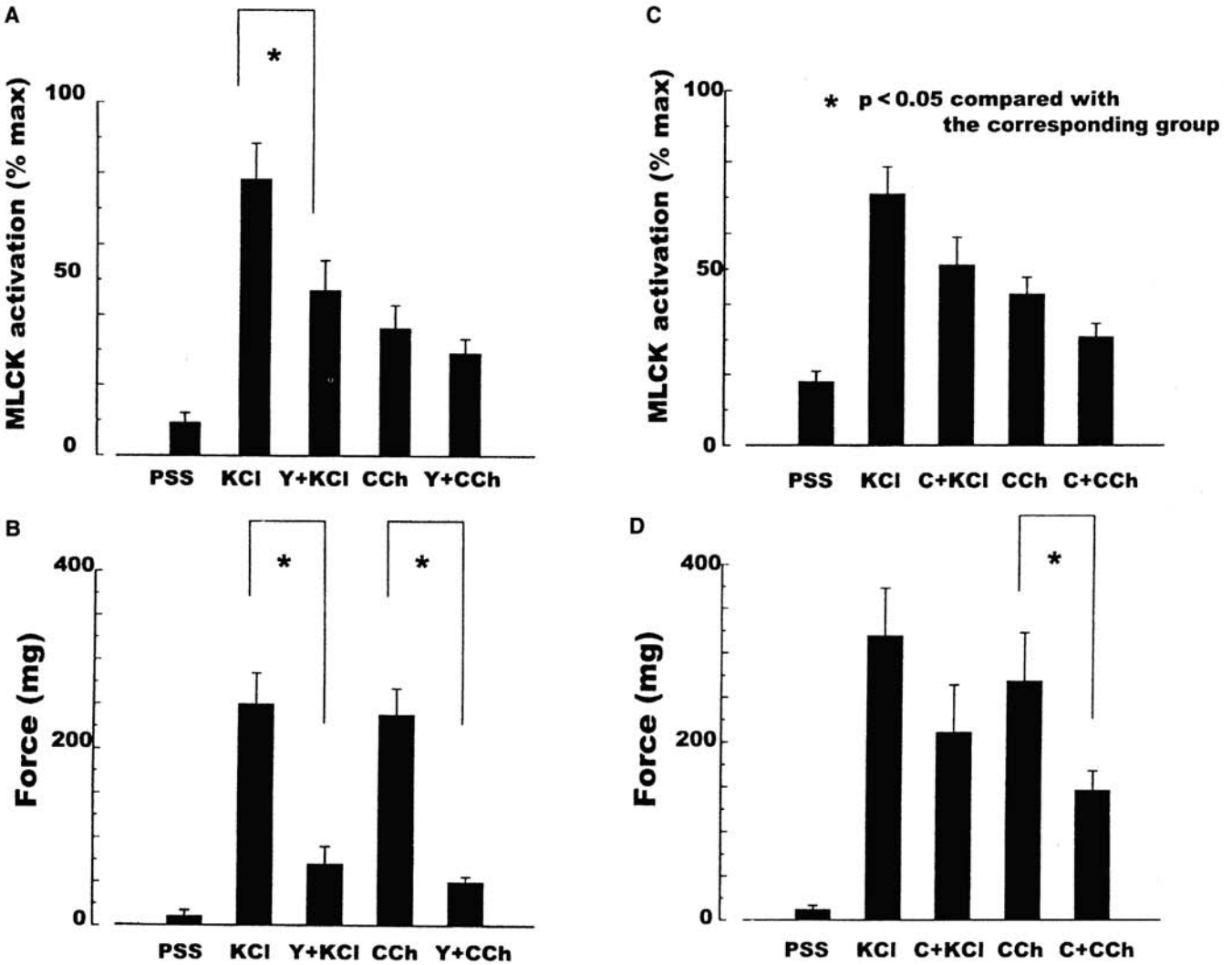


FIGURE 2–3 Effects of Rho kinase inhibitor Y27632 (Y) and protein kinase C inhibitor, calphostin C (C) on FRET and force in intact smooth muscle. (A,B) Y27632 significantly reduces FRET and force in muscle contracted with KCl but only force in muscle contracted with carbachol (CCh). (C,D) The protein kinase C inhibitor reduces only force in muscle contracted with carbachol and has no effect on FRET.

R480/525 and force in skinned smooth muscle at pCa 6.0 plus 0.5 µmol/L calmodulin resulted in 28% and 8% of the respective maximal responses at pCa 3.7. The greater extent of MLC kinase activation at pCa 6.0 relative to force is probably necessary to exceed myosin regulatory light chain phosphatase activity. In KCl-stimulated intact smooth muscle tissue, the R480/525 increased to 58% of the maximal value measured after permeabilization. In agonist-induced contraction, MLC kinase was activated only to 35%. Y27632, a specific Rho kinase inhibitor, almost abolished both KCl-induced contraction and MLC kinase activity. In agonist-stimulated smooth muscle, the contraction was abolished, but MLC kinase activity was not reduced by Y27632. Calphostin C, a protein kinase C inhibitor, did not show any significant inhibition of either contraction or MLC kinase activity (Fig. 2–3).

Discussion

We have demonstrated simultaneous measurements of MLC kinase activity and contraction in smooth muscle tissues. Our results clearly indicate that changes in $[Ca^{2+}]_i$ in the range of less than 1 µmol/L are coupled to changes in the R480/525, but not to the tension developed in skinned fibers. This suggests that the greater MLC kinase activation relative to force needs to overcome myosin regulatory light chain phosphatase activity. In intact smooth muscle tissue, MLC kinase activation has some restriction probably due to the limited concentration of intracel-

lular calmodulin.[3] During agonist-induced contraction, MLC kinase was not activated to the level observed during depolarization-induced contraction. This result is compatible with the phenomenon of Ca^{2+} sensitization that occurs in agonist-induced contraction. A Rho kinase inhibitor had substantial effects on both KCl-induced and agonist-induced contraction, but a protein kinase C inhibitor did not. This suggests that Rho kinase is an important regulator of smooth muscle contraction. It should be noted, however, that the specificity of Y27632 is relative and that this compound also reduces the MLC kinase activity during depolarization. Finally, the contribution of myosin regulatory light chain phosphorylation to cerebral vasospasm has not been clarified. Smooth muscle contraction has at least two different phases that include a rapid, phasic contraction and a delayed, sustained contraction. The former is produced, for example, by serotonin (5-hydroxytryptamine or 5-HT), and the latter is produced by phorbol esters such as phorbol-12,13-dibutyrate in intracranial arteries. Phasic contraction in response to 5-HT-induced contraction can be further divided into Ca^{2+}-dependent and Ca^{2+}-independent pathways. The Ca^{2+}-dependent signaling pathway activates MLC kinase, whereas the Ca^{2+}-independent pathway activates Rho kinase and protein kinase C and leads to inhibition of myosin regulatory light chain phosphatase activity. On the other hand, phorbol-12,13-dibutyrate induces protein kinase activation and a myosin regulatory light chain phosphorylation-independent signaling pathway that can be inhibited by Y27632. The involvement of myosin regulatory light chain phosphorylation and of the Rho kinase and protein kinase C pathways during cerebral vasospasm are the subject of some investigations reported elsewhere in this volume and remain to be fully clarified.

Conclusion

Rho kinase has an important role in both KCl-induced (Ca^{2+}-dependent) and agonist-induced (Ca^{2+}-independent) contractile pathways. MLC kinase was not fully activated during depolarization-induced contraction. Smooth muscle tissues from the mice described herein may provide new insights into the modulation of MLC kinase activity in vivo and to the pathogenesis of vasospasm.

REFERENCES

1. Kamm KE, Stull JT. Activation of smooth muscle contraction: relation between myosin phosphorylation and stiffness. Science 1986;232:80–82
2. Mitra RD, Silva CM, Youvan DC. Fluorescence resonance energy transfer between blue emitting and red-shifted excitation derivatives of the green fluorescent protein. Gene 1996;173:13–17
3. Romoser VA, Hinkle PM, Persechini A. Detection in living cells of Ca^{2+}-dependent changes in the fluorescent protein variants linked by a calmodulin-binding sequence: a new class of fluorescent indicators. J Biol Chem 1997;272:13270–13274

3

Changes in Vascular Ion Channels After Experimental Subarachnoid Hemorrhage

YASUO AIHARA, M.D., PH.D., BABAK S. JAHROMI, M.D., PH.D., REZA YASSARI, M.D., ELENA NIKITINA, PH.D., MAYOWA AGBAJE-WILLIAMS, B.A., R. LOCH MACDONALD, M.D., PH.D.

Abstract

There is evidence that smooth muscle cells are depolarized during vasospasm after subarachnoid hemorrhage (SAH). This study tested whether alterations in ion channels at the level of messenger ribonucleic acid (mRNA) and/or protein occur in these cells and could contribute to vasospasm. Expression of mRNA and protein for ion channels in the basilar arteries of dogs after SAH was assessed using quantitative real-time polymerase chain reaction (PCR) and western blotting. SAH was associated with vasospasm of the basilar artery (41 ± 8% reduction in arterial diameter, $p < .001$) at 7 days. There was a significant decrease in mRNA and protein for the voltage-gated K+ channel K_v 2.2 (65% reduction in mRNA, $p < .001$; 49% reduction in protein, $p < .05$). The mRNA of the ß1 subunit of the large- or big-conductance, Ca^{2+}-activated K+ (BK) channel also was significantly reduced (53% reduction in mRNA, $p < .02$) whereas protein for this channel was not. The mRNA and protein for an inwardly rectifying K+ (K_{ir}) channel K_{ir} 2.1 were upregulated (234% increase in mRNA, $p < .001$; 350% increase in protein, $p < .001$). There was no significant change in mRNA expression of L-type Ca^{2+} channels and the BK-a subunit. These alterations in K+ channels may contribute to the pathogenesis of cerebral vasospasm.

Prior experiments from our laboratory suggest that there is increasing impairment of smooth muscle relaxation as vasospasm progresses after SAH.[1] Relaxation of smooth muscle occurs via several pathways including one mediated by nitric oxide (NO) produced in endothelial cells by endothelial NO synthase (NOS) that leads to an increase in cyclic guanosine monophosphate in vascular smooth muscle. Relaxation ultimately occurs in part by activation of cyclic guanosine monophosphate–dependent protein kinase and potentiation of BK channels[2] and inhibition of L-type Ca^{2+} channels.[3] We investigated the proximal portion of this pathway and found that vasospasm could not be prevented by NO donors, suggesting that a defect in the vasodilatory pathway distal to NO is important.[4] Review of the literature provides circumstantial evidence for such an abnormality in vasospasm. For example, vasospastic smooth muscle cells have been reported to be depolarized relative to controls after experimental SAH, although the exact basis for this has not been

clarified.[5–7] The experiments herein were performed to determine if changes in ion channels that regulate membrane potential might occur after SAH and be at least a sufficient cause for the depolarization.

Materials and Methods

The double hemorrhage dog model of SAH was performed as described.[8] All procedures performed on animals were approved by the Institutional Animal Care and Use Committee. Seventeen animals with SAH, were euthanized under general anesthesia by exsanguination and perfusion with ice-cold phosphate-buffered saline (PBS, pH 7.4) 7 days after the first SAH but an additional 17 control animals did not undergo SAH and were simply exsanguinated and perfused. The basilar arteries were carefully isolated and frozen in liquid nitrogen and then used for RNA and protein extraction with TRIzol (Gibco BRL; Life Technologies, Rockville, MD, USA). RNA reverse transcription was catalyzed using a SUPER SCRIPT First-Strand Synthesis System (Invitrogen Life Technologies, Carlsbad, CA, USA), and protein was quantified spectrophotometrically (Micro BCA Protein Assay Reagents, Pierce Biotechnology, Rockford, IL, USA). Quantitative PCR was conducted with real-time TaqMan technology using a Sequence Detection System model 7700 (Applied Biosystems, Foster City, CA).[9] Dog mRNA sequences were obtained from the National Center for Biotechnology Information (NCBI) database (http://www.ncbi.nlm.nih.gov/Entrez). Expression levels of target genes were compared with 18S rRNA. Western blotting of proteins was performed on Hybond™ P (polyvinylidene difluoride) membranes exposed to primary antibodies including rabbit anti-BK-pl polyclonal antibody (1:500 dilution, Affinity Bioreagents, Golden, CO, USA), mouse monoclonal anti-K_v 2.2 antibody (1:1000 dilution, obtained from J.S. Trimmer, State University of New York, Stonybrook, NY, USA), and rabbit antihuman K_{ir} 2.1 antibody (1:200 dilution, Alomone Laboratories, Jerusalem, Israel). Secondary antibodies were goat antirabbit alkaline phosphate-linked antibody (1:5000, Amersham Biosciences, Piscataway, NJ, USA) for BK-ß1, horseradish peroxidase-conjugated antimouse antibody (1:2000 dilution, Santa Cruz Biotechnology, Santa Cruz, CA, USA) for K_v 2.2, and horseradish peroxidase-conjugated IgG antirabbit antibody (1:2500 dilution, Cell Signaling Technology, Beverly, MA, USA) for K_{ir} 2.1. Detection was by enhanced chemifluorescence (Amersham Bioscience, Arlington Heights, IL, USA) and a Storm 860 fluorimager (Molecular Dynamics, Sunnyvale, CA, USA) for BK-ß1 and enhanced chemiluminescence Plus western blotting detection system (Amersham) for K_v 2.2 and K_{ir} 2.1.

Results

The basilar artery diameter decreased on day 7 to 41 ± 8% of the pre-SAH diameter ($p < .001$, paired *t*-test). Each target gene mRNA was expressed as a ratio of its C_T value to that of 18S rRNA, and these normalized ratios were then compared between control and SAH dogs. The dog basilar artery contained mRNA for the BK channel including the pore-forming a subunit and a regulatory ß subunit.[10] The ß subunit was the ß1 isoform, as would be expected. SAH was associated with a significant reduction in BK-ß1 subunit compared with control (53% reduction, $p < .02$, Fig. 3–1), whereas there was no change in the BK-a subunit. Other mRNAs for ion channels that were not significantly altered by SAH included those for the L-type Ca^{2+} channel and multiple K_v channels (K_v 1.2, K_v 1.5, K_v 3.1, and K_v 4.3). There was, however, a significant reduction in K_v 2.2 and K_v 3.4 mRNA (65% and 38%, respectively, $p < .001$ and $p < .05$, unpaired *t*-tests, see Fig. 3–1) and a significant increase in K_{ir} 2.1 (234%, $p < .001$; see Fig. 3–1).

Western blots were normalized by loading identical amounts of total protein per lane. Bands consistent with BK-ß1 subunit (~ 28 kD), K_v 2.2 (~ 98 kD), and K_{ir} 2.1 (~ 60 kD, Fig. 3–2) were detected. The results were consistent with the changes in mRNA except that BK-ß1 subunit expression was unchanged between the SAH and control group (data not shown). K_v 2.2 and K_{ir} 2.1 protein levels were altered in the same manner as mRNA for these genes. K_{ir} 2.1 protein was significantly increased (350% increase, $p < .001$, unpaired *t*-test) and K_v 2.2 protein significantly decreased (49% decrease, $p < .05$, unpaired *t*-test) in SAH compared with control basilar arteries (see Fig. 3–2).

Discussion

Cerebrovascular smooth muscle cells are known to possess K_{ir}, adenosine-triphosphate-sensitive K+, K_v, and BK channels. The latter two are of key importance in regulating the diameter of cerebral arteries.[11] K+ channels exert their effect in part by controlling smooth muscle membrane potential. Small changes in membrane potential that can result from changes in K+ channel function may exert large effects on vascular tone.[12] There is indirect evidence for an alternation in K+ channels in vasospasm in that smooth muscle cells of dogs with SAH showed resting membrane potentials that were 10 to 20 mV depolarized with respect to control animals.[5] In addition, K+ channel agonists such as cromakalim[7,13,14] and nicorandil[15] partially reduce experimental vasospasm. In this study, dog basilar arteries were found to contain mRNA and protein for BK channels and a variety of K_v channels.

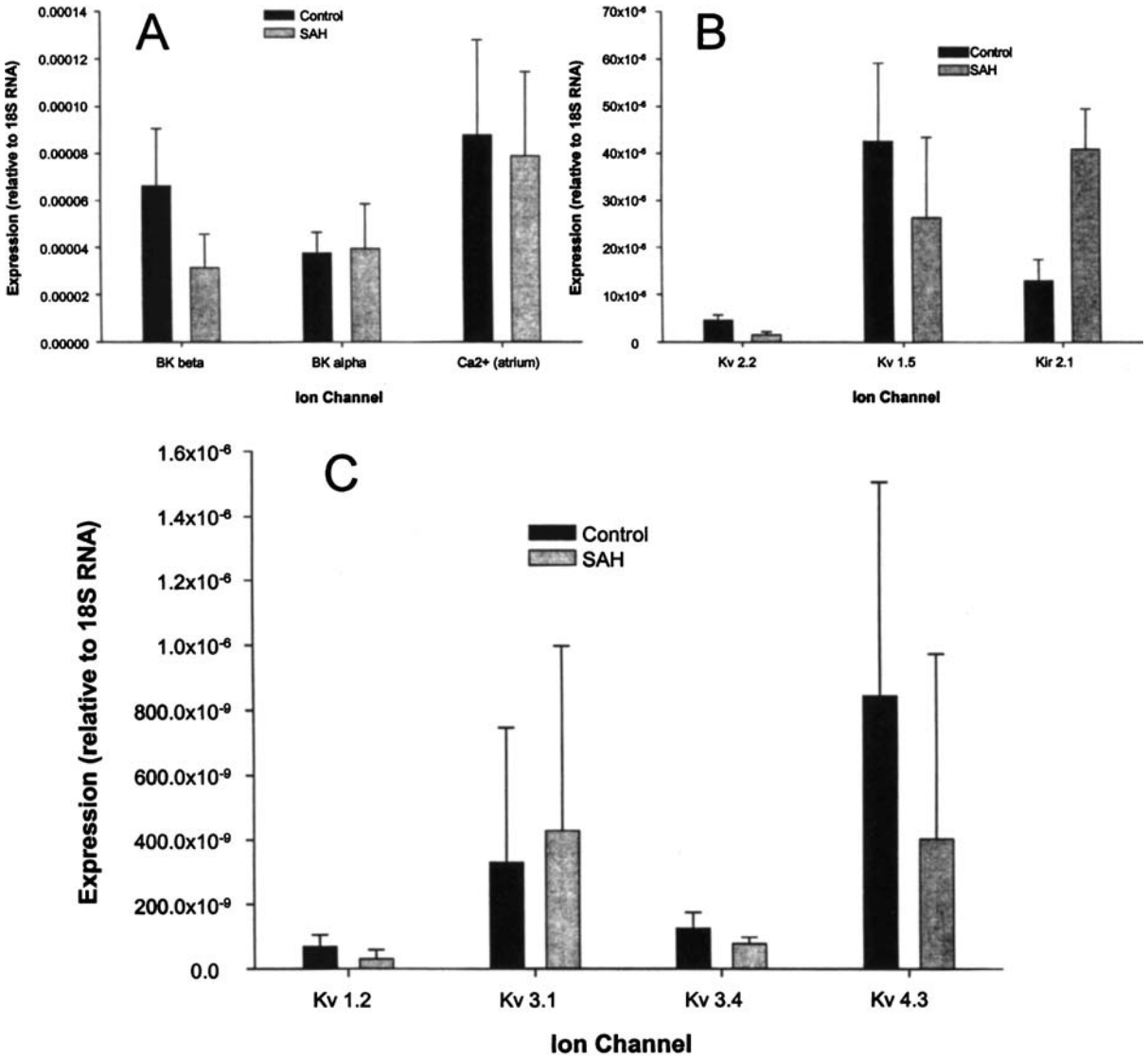


FIGURE 3–1 (A) Real-time quantitative polymerase chain reaction of mRNA expression of large-conductance, Ca^{2+}-activated K+ (BK) a and ß1 subunits and the a subunit of the Ca^{2+} L-type channel; (B) K_v 1.5 and 2.2 and K_{ir}; and (C) K_v 1.2, 3.1, 3.4, and 4.3. Levels are expressed relative to 18S RNA. There was significant reduction in expression of the regulatory ß1 subunit of the BK channel and of K_v 2.2 and K_v 3.4 and a significant increase in K_{ir} after subarachnoid hemorrhage (n = 6–7 per group, $p < .05$, unpaired t-test).

The BK channel generally mediates vascular relaxation because it is activated by membrane depolarization and raised $[Ca^{2+}]_i$. This leads to outward, hyperpolarizing K+ current that closes L-type Ca^{2+} channels and reduces $[Ca^{2+}]_i$.[12] The present results do not support a major role for alterations in this channel during vasospasm.

Evidence was found, however, for a role of K+ channels of the K_v family in vasospasm. The exact isoforms present probably vary depending on the arterial bed and species examined, although these channels are important in regulating cerebrovascular tone as shown by vasoconstrictor responses to 4-aminopyridine (a K_v blocker) that occur in cerebral arteries.[16,17] Although the function of the numerous isoforms identified in dog basilar artery is unknown at present, reduced K_v mRNA and protein that were observed in vasospastic arteries in this study could result in reduced baseline K+ conductance and ability to repolarize following vasoconstrictive stimuli, both of which also could contribute to vasospasm. The third K+ channel identified was a K_{ir} type. These channels are believed to mediate cerebral vasodilation that follows activity-mediated increases in extracellular K+.[18] The increase in K_{ir} 2.1 mRNA and protein observed in vasospastic arteries in this study may represent a compensatory mechanism

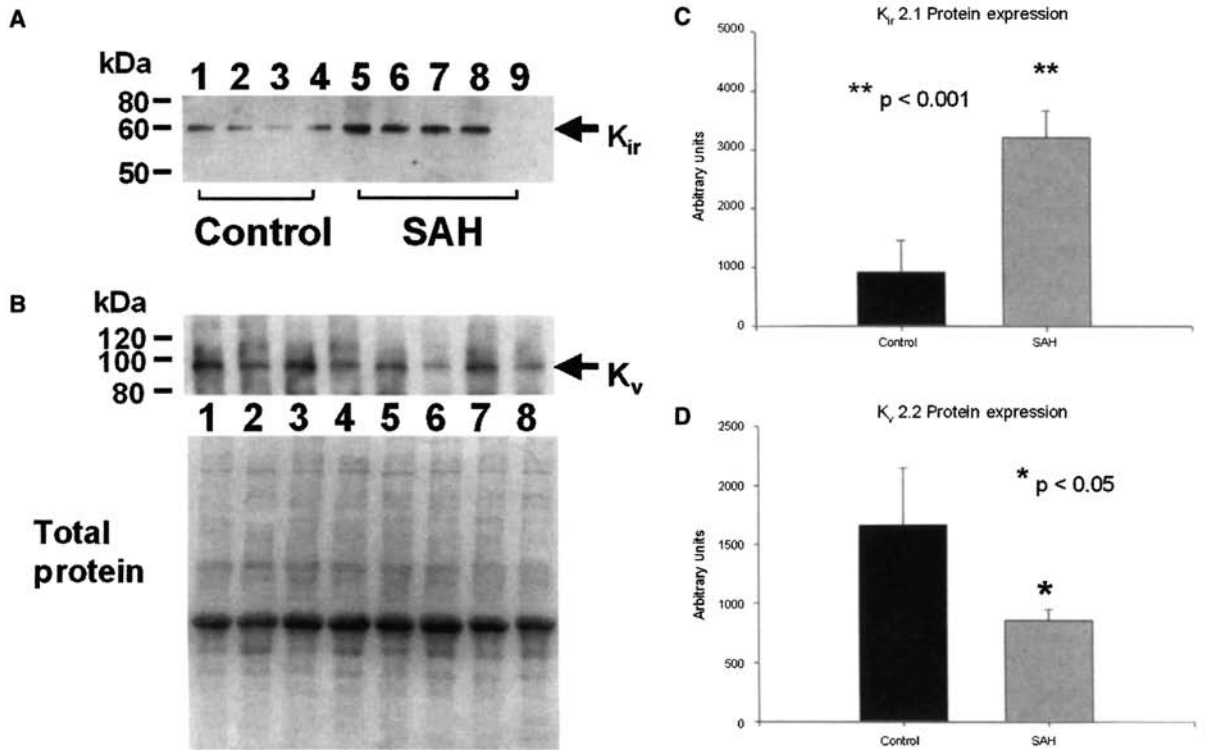


FIGURE 3–2 Western blot analysis of K_{ir} 2.1 and K_v 2.2 expression in the dog basilar artery. (A) K_{ir} 2.1 channel protein (~ 60 kD) is greater in subarachnoid hemorrhage (SAH) group (lanes 5 to 8) than control group (lanes 1 to 4), and no signal is present when using a blocking peptide (lane 9). (B) Protein levels of K_v 2.2 (~ 98 kD) are downregulated in SAH (lanes 2,4,6,8) as compared with control basilar artery (lanes 1, 3, 5, 7). Total protein extracts loaded in each lane were visualized by staining the transferred PVDF membrane with GelCode Blue Stain Reagent (Pierce Biotechnology). Each lane contains an equal amount of total protein. (C) Quantification of bands by photometric analysis shows significant upregulation of K_{ir} 2.1 and (D) downregulation of K_v 2.2 (n = 4–8 per group, unpaired t-tests).

directed against the sustained smooth muscle cell contraction and the decreased K_v 2.2 and BK-ß1 expression observed.

Some limitations of the present work should be acknowledged. Relationships between alterations in ion channel mRNA and protein and vasospasm were identified, although these results do not indicate whether the observed changes are a cause or a consequence of SAH. Furthermore, the functional importance of these changes is not known and would need to be determined because small changes in either or both mRNA and protein may or may not be sufficient to alter K+ channel function and membrane potential. For example, it is interesting that during vasospasm there was an increase in K_{ir} 2.1. This channel mediates relaxation of arteries, yet the increase occurs during the constriction of vasospasm.

Acknowledgment

This work was supported by grants (to R.L.M.) from the American Heart Association, National Institutes of Health (NS25946), and Brain Research Foundation. B.S.J, was supported by the Canadian Institutes of Health Research and the American Association of Neurological Surgeons. We thank Dr. J.S. Trimmer (State University of New York, Stonybrook NY, USA) for providing K_v 2.2 antibody.

REFERENCES

1. Zhang ZD, Yamini B, Komuro T, et al. Vasospasm in monkeys resolves because of loss of and encasement of subarachnoid blood clot. Stroke 2001;32:1868–1874
2. Robertson BE, Schubert R, Hescheler J, Nelson MT. cGMP-dependent protein kinase activates Ca-activated K channels in cerebral artery smooth muscle cells. Am J Physiol 1993;265: C299–C303
3. Simard JM, Li X, Tewari K. Increase in functional Ca^{2+} channels in cerebral smooth muscle with renal hypertension. Circ Res 1998;82:1330–1337
4. Macdonald RL, Zhang ZD, Curry D, et al. Intracisternal sodium nitroprusside fails to prevent vasospasm in nonhuman primates. Neurosurgery 2002;51:761–768
5. Harder DR, Dernbach P, Waters A. Possible cellular mechanism for cerebral vasospasm after experimental subarachnoid hemorrhage in the dog. J Clin Invest 1987;80:875–880

6. Waters A, Harder DR. Altered membrane properties of cerebral vascular smooth muscle following subarachnoid hemorrhage: an electrophysiological study, I: Changes in resting membrane potential (Em) and effect on the electrogenic pump potential contribution to Em. Stroke 1985;16:990–997

7. Zuccarello M, Bonasso CL, Lewis AI, Sperelakis N, Rapaport RM. Relaxation of subarachnoid hemorrhage–induced spasm of rabbit basilar artery by the K+ channel activator cromakalim. Stroke 1996;27:311–316

8. Macdonald RL, Zhang J, Sima B, Johns L. Papaverine-sensitive vasospasm and arterial contractility and compliance after subarachnoid hemorrhage in dogs. Neurosurgery 1995;37:962–967

9. Aihara Y, Kasuya H, Onda H, Hori T, Takeda J. Quantitative analysis of gene expressions related to inflammation in canine spastic artery after subarachnoid hemorrhage. Stroke 2001;32:212–217

10. Brenner R, Perez GJ, Bonev AD, et al. Vasoregulation by the beta1 subunit of the calcium-activated potassium channel. Nature 2000;407:870–876

11. Faraci FM, Sobey CG. Role of potassium channels in regulation of cerebral vascular tone. J Cereb Blood Flow Metab 1998;18: 1047–1063

12. Knot HJ, Nelson MT. Regulation of arterial diameter and wall [Ca^{2+}] in cerebral arteries of rat by membrane potential and intravascular pressure. J Physiol 1998;508:199–209

13. Kwan AL, Lin CL, Wu CS, et al. Delayed administration of the K+ channel activator cromakalim attenuates cerebral vasospasm after experimental subarachnoid hemorrhage. Acta Neurochir (Wien) 2000;142:193–197

14. Sugai K, Yaganisawa T, Motohashi O, Suzuki M, Yoshimoto T. Levcromakalim decreases cytoplasmic Ca^{2+} and vascular tone in basilar artery of SAH model dogs. J Cardiovasc Pharmacol 1999;33:868–875

15. Matsui T, Nagafuji T, Tsutsumi K, Uchida H, Miyauchi T, Asano T. The effect of nicorandil on chronic cerebral vasospasm. Acta Neurochir (Wien) 1994;126:165–169

16. Knot HJ, Nelson MT. Regulation of membrane potential and diameter by voltage-dependent K+ channels in rabbit myogenic cerebral arteries. Am J Physiol 1995;269:H348–H355

17. Quan L, Sobey CG. Selective effects of subarachnoid hemorrhage on cerebral vascular responses to 4-aminopyridine in rats. Stroke 2000;31:2460–2465

18. Edwards FR, Hirst GD, Silverberg GD. Inward rectification in rat cerebral arterioles; involvement of potassium ions in autoregulation. J Physiol 1988;404:455–466

4

Inhibition of Src Tyrosine Kinase Reduces Experimental Cerebral Vasospasm

GEN KUSAKA, M.D., HITOSHI KIMURA, M.D., IKUYO KUSAKA, M.D., PH.D., EDDIE PERKINS, M.S., ANIL NANDA, M.D., JOHN H. ZHANG, M.D., PH.D.

Abstract

We investigated whether inhibition of Src tyrosine kinase would reduce experimental cerebral vasospasm in dogs. Vehicle [dimethylsulfoxide (DMSO)] or the Src inhibitors 4-amino-5-(4-chlorophenyl)-7-(t-butyl)pyrazolo[3,4-d] pyrimidine (PP2) or damnacanthal were injected into the cisterna magna daily from day 2 through day 6. Severe vasospasm was observed in the basilar arteries of vehicle-treated dogs. Mild vasospasm was observed in all dogs treated with Src inhibitors. Phosphorylated Src and mitogen-activated protein (MAP) kinase were increased after subarachnoid hemorrhage (SAH), and the activation of these kinases was abolished by PP2 and damnacanthal in the basilar arteries. Inhibition of Src might offer a new therapy in the management of cerebral vasospasm.

We have shown previously that spasmogens such as endothelin and erythrocyte hemolysate modulate extracellular regulated kinase (ERK)-1/2 activity.[1–4] We propose that Src family nonreceptor tyrosine kinases are important upstream regulators of ERK-1/2 and that alterations in Src activation could underlie abnormal signaling by spasmogens in cerebral vasospasm after SAH. Downstream targets of Src include p21Ras, which activates MAP kinase kinase 1/2 (MEK1/2), and, in turn, phosphorylates ERK1/2.[5] Many Src-family tyrosine kinases have been identified. Of these, the 60 kDa c-Src is the prototype.[5]

Materials and Methods

Twenty-four adult mongrel dogs of either sex, weighing 20 to 25 kg, were used either for the double-hemorrhage model or as normal controls as described previously.[1,6–8] The dogs were randomly divided into four groups to undergo treatment with drug vehicle (DMSO, $n = 6$), damnacanthal ($n = 6$), or PP2 ($n = 6$). One group ($n = 6$) was not subjected to SAH or treated with drugs and served as the normal control group. Treatment was administered by intracisternal injection and began on day 2 at the time of the second blood injection into the cisterna magna and ended on day 6. Arterial diameters were measured in a double-blind fashion on magnified angiograms.[1,8,9] The method for Western blotting of canine basilar arteries has been described previously.[2,6–8]

Data are expressed as means ± standard error of the mean (SEM). Statistical differences between the control and other groups were compared by one-way analysis of variance (ANOVA) and then the Tukey-Kramer multiple comparison procedure if a significant difference had been determined by ANOVA. A probability value of $p < .05$ was considered statistically significant.

Results

In the vehicle-treated group, the mean value of the residual diameter of the basilar artery on day 7, as a percentage of that on day 0, was 42.6 ± 1.5%. In the PP2- and damnacanthal-treated groups, the mean residual diameters of the basilar arteries on day 7 were 83.2 ± 2.9% and 75.7 ± 4.4% of day 0, respectively. The percent reductions in diameters in these groups were significantly less than that of the DMSO-treated group ($p < .05$, ANOVA). The activation of Src and MAP kinase in normal dogs was normalized and used as 100% to compare with activation in each of the SAH groups. In the DMSO-treated group, Src and MAP kinase activation was significantly increased by SAH on day 7 (150% and 157%, respectively) compared with the basilar arteries in normal dogs with no SAH ($p < .05$ vs vehicle, ANOVA). Treatment with PP2 significantly suppressed the activation of Src and MAP kinase (90% and 95%, respectively, $p < .01$ vs vehicle group, ANOVA). Similarly, damnacanthal significantly suppressed activation of these kinases (90% and 99%, respectively, $p < .01$ vs vehicle group, ANOVA).

Discussion

We have demonstrated in the present study that phosphorylated Src and MAP kinase are increased in the vasospastic canine basilar artery 7 days after experimental SAH. The intracisternal administration of Src inhibitors, PP2 or damnacanthal, abolished the activation of Src and MAP kinases and concomitantly attenuated angiographic vasospasm. The Src family-selective tyrosine kinase inhibitors, PP2 and damnacanthal, have been employed as powerful tools for the investigation of molecular and cellular mechanisms of physiological and pathophysiological events associated with the activation of the Src family of tyrosine kinases.[10] PP2 is known to inhibit the Src family of tyrosine kinases at submicromolar concentrations in vitro and at low micromolar concentrations in intact cells without affecting other protein tyrosine kinase and receptor tyrosine kinase activities.[11] Damnacanthal also is known to be an Src family inhibitor and has been used in previous studies in our laboratory.[2] In the present study, injection of PP2 and damnacanthal into the cisterna magna abolished the enhanced Src and MAP kinase activity in the basilar artery that occurred 7 days after experimental SAH. Furthermore, this effect was associated with a reduction in cerebral vasospasm. These results suggest that Src-dependent MAP kinase pathways contribute to cerebral vasospasm after SAH.

MAP kinases are a group of serine/threonine kinases that are activated by a cascade of protein kinases to induce biological responses including smooth muscle contraction. Key components of the ERK pathway include the small GTPase Ras, the cytosolic serine/threonine kinase Raf-1 (MAP kinase kinase kinase), and MEK1/2 (MAP kinase kinase). MAP kinase kinase phosphorylates and activates ERK1/2. MAP kinase was activated in cerebral arteries during cerebral vasospasm after SAH.[1–4,12,13] This activation may be mediated by Src, Ras, Rho, and other tyrosine kinases[12,14–17] following the activation of either growth factor receptors or G-protein coupled receptors. Src might play a key role in mediating signaling events from the activation of receptors to the activation of MAP kinase during cerebral vasospasm.

Cerebral vasospasm probably is caused by multiple factors including both agonist-mediated receptor activation and stimulation of nonreceptor pathways. The main component of the subarachnoid blood clot is hemoglobin, including at various times after SAH oxyhemoglobin, deoxyhemoglobin, and methemoglobin. Hemoglobin breaks down into different metabolites, including bilirubin, heme, hemin, globin, and free iron. All of these products of hemoglobin have been shown to play a role in cerebral vasospasm.[18] All these factors might activate different pathways that are linked with Src kinase in cerebral arteries. Activation of Src leads to the activation of MAP kinase, which we hypothesize contributes to cerebral vasospasm. Our data raise the possibility that Src family inhibitors may be candidates for treating cerebral vasospasm after SAH.

Acknowledgments

This work was partially supported by grants from the American Heart Association Bugher Foundation Award for Stroke Research and the National Institutes of Health (NS45694) to J.H.Z.

REFERENCES

1. Tibbs R, Zubkov A, Aoki K, et al. Effects of mitogen-activated protein kinase inhibitors on cerebral vasospasm in a double-hemorrhage model in dogs. J Neurosurg 2000;93:1041–1047
2. Zubkov AY, Ogihara K, Patllola A, Parent AD, Zhang J. Mitogen-activated protein kinase plays an important role in hemolysate-induced contraction in rabbit basilar artery. Acta Neurochir Suppl 2000;76:217–221
3. Zubkov AY, Rollins KS, McGehee B, Parent AD, Zhang JH. Relaxant effect of U0126 in hemolysate-, oxyhemoglobin-, and bloody cerebrospinal fluid-induced contraction in rabbit basilar artery. Stroke 2001;32:154–161
4. Zubkov AY, Rollins KS, Parent AD, Parent AD, Zhang J, Bryan RM. Mechanism of endothelin-1-induced contraction in rabbit basilar artery. Stroke 2000;31:526–533
5. Ishida M, Ishida T, Thomas SM, Berk BC. Activation of extracellular signal-regulated kinases (ERK1/2) by angiotensin II is dependent on c-Src in vascular smooth muscle cells. Circ Res 1998;82:7–12
6. Satoh M, Parent AD, Zhang JH. Inhibitory effect with antisense mitogen-activated protein kinase oligodeoxynucleotide against cerebral vasospasm in rats. Stroke 2002;33:775–781

7. Kimura H, Meguro T, Badr A, Zhang JH. Suramin-induced reversal of chronic cerebral vasospasm in experimental subarachnoid hemorrhage. J Neurosurg 2002;97:129–135
8. Kimura H, Sasaki K, Meguro T, Zhang JH. Phosphatidylinositol 3-kinase inhibitor failed to reduce cerebral vasospasm in dog model of experimental subarachnoid hemorrhage. Stroke 2002;33:593–599
9. Satoh M, Perkins E, Kimura H, et al. Posttreatment with adenovirus-mediated gene transfer of calcitonin gene-related peptide to reverse cerebral vasospasm in dogs. J Neurosurg 2002;97:136–142
10. Hanke JH, Gardner JP, Dow RL, et al. Discovery of a novel, potent, and Src family-selective tyrosine kinase inhibitor: study of Lck- and FynT-dependent T cell activation. J Biol Chem 1996;271:695–701
11. Blake RA, Garcia-Paramio P, Parker PJ, Courtneidge SA. Src promotes PKCdelta degradation. Cell Growth Differ 1999;10:231–241
12. Vollrath B, Cook D, Megyesi J, Findaly JM, Ohkuma H. Novel mechanism by which hemoglobin induces constriction of cerebral arteries. Eur J Pharmacol 1998;361:311–319
13. Fujikawa H, Tani E, Yamaura I, et al. Activation of protein kinases in canine basilar artery in vasospasm. J Cereb Blood Flow Metab 1999;19:44–52
14. Sato M, Tani E, Fujikawa H, Kaibuchi K. Involvement of Rho-kinase-mediated phosphorylation of myosin light chain in enhancement of cerebral vasospasm. Circ Res 2000; 87:195–200
15. Patlolla A, Zubkov A, Parent A, Zhang J. Hemolysate activates P21RAS in rabbit basilar artery. Life Sci 2000;67:1233–1242
16. Patlolla A, Ogihara K, Aoki K, et al. Hemolysate induces tyrosine phosphorylation and collagen-lattice compaction in cultured fibroblasts. Biochem Biophys Res Commun 1999;264:100–107
17. Miyagi Y, Carpenter RC, Meguro T, Parent AD, Zhang JH. Upregulation of rho A and rho kinase messenger RNAs in the basilar artery of a rat model of subarachnoid hemorrhage. J Neurosurg 2000;93:471–476
18. Macdonald RL, Weir BK. A review of hemoglobin and the pathogenesis of cerebral vasospasm. Stroke 1991;22:971–982

5

Potassium Channels in Experimental Cerebral Vasospasm

BABAK S. JAHROMI, M.D., PH.D., YASUO AIHARA, M.D., PH.D., REZA YASSARI, M.D., ELENA NIKITINA, PH.D., DEVAN RYAN, B.S., GEORGE WEYER, MAYOWA AGBAJE-WILLIAMS, B.A., R. LOCH MACDONALD, M.D., PH.D.

Abstract

It has been shown that vasospastic smooth muscle cells are depolarized relative to normal smooth muscle cells. This membrane depolarization could contribute to smooth muscle contraction, which is one of the underlying processes that occurs in cerebral vasospasm. Membrane potential of smooth muscle is determined largely by K+ conductance. This study tested the hypothesis that alteration in K+ channel conductance underlies membrane depolarization in vasospastic smooth muscle. Dogs were used as controls or underwent creation of subarachnoid hemorrhage using the double hemorrhage model. Smooth muscle cells were isolated from the basilar arteries and used for electrophysiological studies. Membrane potential was measured in control and vasospastic cells. The major K+ channels contributing to membrane potential in these cells were large conductance, Ca^{2+}-activated K+ (BK), and voltage-gated K+ (Kv) channels. Vasospastic myocytes were depolarized by ~9.5 mV compared with control cells. The current density, voltage-dependence, and Ca^{2+} sensitivity of the BK channel in control and vasospastic myocytes were compared using whole-cell patch-clamp methods. No differences in BK current density or Ca^{2+} and voltage sensitivity were observed between control and vasospastic myocytes. On the other hand, Kv current density was nearly halved in vasospastic myocytes after subarachnoid hemorrhage (SAH). The results suggest that Kv channel dysfunction may contribute to cerebral vasospasm.

Sharp microelectrode recordings from cat middle cerebral artery at different transmural pressure gradients demonstrate that there is a direct relationship between the membrane potential of arterial smooth muscle cells and the intraluminal pressure.[1] In addition, increasing intra-arterial pressure causes depolarization, which is followed by a rise in intracellular Ca^{2+} ($[Ca^{2+}]_i$) and arterial constriction.[2] The increase in $[Ca^{2+}]_i$ and constriction but not the depolarization are blocked by voltage-gated Ca^{2+} channel blockers. Thus, membrane potential is an important modulator of cerebral arterial diameter and K+ currents, and the K+ channels through which they pass are important regulators of membrane potential in arterial smooth muscle.[3] Opening K+ channels will hyperpolarize and closing K+ channels will depolarize smooth muscle. Of the four types of K+ channels that have been found in arterial smooth muscle, key regulators of membrane

potential of large cerebral arteries are the BK and the Kv K+ channels.

Waters and Harder reported that cat basilar artery myocytes were contracted and depolarized by ~15 mV after SAH.[4] They confirmed this observation in the dog double hemorrhage SAH model and, in addition, showed indirectly that K+ conductance was reduced in the depolarized smooth muscle cells and that this could be increased to near control levels with nicorandil, a nonspecific K+ channel opener.[5] Other investigations also have suggested that vasospastic smooth muscle cells are depolarized relative to control cells and that this may be due to alteration in some type of K+ current.[6] The work described herein was designed to test the hypothesis that K+ channel dysfunction contributes to vasospasm in a dog model of SAH.

Materials and Methods

Animal Model

All procedures were approved by the Animal Care and Use Committee of the University of Chicago. Mongrel dogs weighing 15 to 20 kg were randomly allocated to be used as controls or to undergo induction of SAH.[7] Dogs were placed under general anesthesia and underwent baseline cerebral angiography on day 0. Control dogs were sacrificed immediately following angiography. SAH was created using the double hemorrhage model, as previously described.[7] The cisternal injections consisted of 0.5 mL/kg of fresh, autologous, arterial, nonheparinized blood and were administered on day 0 and day 2. Angiography was repeated in these dogs on day 7, and vasospasm was assessed by comparing diameters of basilar arteries from day 0 and day 7.

Tissue Harvest

Animals were euthanized under general anesthesia by exsanguination and perfusion with ice-cold phosphate-buffered saline (pH 7.4) at an intraluminal pressure of 25 to 30 mmHg. The brain was removed, and the basilar and anterior spinal arteries were isolated. The arteries were cut into small pieces and digested in a solution containing 500 units/mL collagenase type IV, 50 units/mL elastase, 100 units/mL DNase I, and 1 mg/mL trypsin inhibitor (Worthington, Lakewood, NJ, USA). Smooth muscle cells were released by gentle trituration of the digested arterial fragments.

Electrophysiology

Macroscopic currents were recorded using the whole-cell patch-clamp method.[8] Currents were amplified using an Axopatch-1D amplifier (Axon Instruments, Union City, CA, USA) and digitized at 12 bits using a DigiData 1200 interface controlled by pClamp 6.0.4 (Axon Instruments). Series resistance was usually < 5 MO and was always compensated by = 80%. All recordings were at room temperature (22°C). Standard extracellular solution contained (in mmol/L): NaCl 145, KCl 5, $MgCl_2$ 1, $CaCl_2$ 2, $CdCl_2$ 0.2, N-[2-hydroxyethyl]piperazine-N'[2-ethanesulfonic acid] (HEPES) 10, glucose 10, pH 7.4. BK currents were recorded using an intracellular solution of (in mmol/L): KCl 20, choline-Cl 120, $MgCl_2$ 1.5, $CaCl_2$ 2.6, ethylenebis (oxonitrilo)tetraacetic acid (EGTA) 5, HEPES 5, glucose 5, pH 7.2 with KOH. K+ was replaced with choline to reduce current magnitude.[9] Under these conditions, outward K+ currents were dominated by BK current, as demonstrated by near complete block of such currents by 1 µmol/L paxilline.[10] To determine the Ca^{2+} sensitivity of macroscopic currents, intracellular solutions contained (in mmol/L): KOH 5, choline-Cl 135, $MgCl_2$ 1.5, EGTA 2, HEPES 5, glucose 5, pH 7.2 with KOH and Ca^{2+} added to result in 13, 100, 200, or 500 nmol/L free Ca^{2+}. The external solution contained Cd^{2+} (200 µumol/L) to block L-type Ca^{2+} channels. Tail-current protocols were used to avoid BK channel block by internal divalent cations such as Ba^{2+} and Mg^{2+}.[11]

Kv currents were isolated from BK currents by strong intracellular Ca^{2+} buffering and block of L-type Ca^{2+} channels by external Cd^{2+}. The intracellular solution for recording Kv currents contained (in mmol/L): KCl 88.6, KOH 6.4, K_2ATP 5, NaGTP 0.1, 1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid (BAPTA, tetrapotassium salt) 10, $MgCl_2$ 2.5, HEPES 10, pH 7.2 with KOH. Resulting currents were insensitive to paxilline (1 µmol/L) and glyburide (10 µmol/L). Membrane potential was measured using the nystatin-perforated patch-clamp method in the current-clamp mode. The pipette solution contained (in mmol/L): KCl145, NaCl 5, $MgCl_2$ 1, HEPES 10, pH 7.2 adjusted with KOH. All data are presented as means ± standard error. Student's *t*-test was used to determine significance, which was set at $p < .05$.

Results

Vasospasm, Membrane Potential, and Electrophysiological Characteristics

A subset of dogs subjected to SAH demonstrated that the basilar artery 7 days after SAH was reduced to 53 ± 4% of its diameter on day 0 ($n = 10$, $p < .05$). Vasospastic cells were significantly smaller than control cells (cell membrane capacitance for SAH cells = 27.5 ± 0.7 versus 21.2 ± 0.7 pF for control cells, $n = 29$ for both groups, $p < .01$). The resting membrane potential of vasospastic myocytes (–27 ± 1 mV, $n = 40$) in

physiological $[K+]_o$ was significantly depolarized ($p < .001$) by ~9.5 mV when compared with that observed in control myocytes (–37 ± 1, $n = 57$).

BK Channels

Tail current amplitudes were used to determine steady-state conductance-voltage (GV) relationships.[12] Half-activation potentials ($V_{1/2}$) and slope factors (k) were similar between control and vasospastic myocytes ($V_{1/2}$ = 83 ± 1 vs 81 ± 1 mV and k = 14 ± 1 vs 15 ± 1 mV per *e*-fold change in membrane potential, respectively, $n = 29$ for both groups, Fig. 5–1). There was no significant difference in the current density of control versus vasospastic myocytes. The BK channel may be opened by either Ca^{2+} or depolarization, with either factor reducing the apparent activation threshold for the other.[13] The Ca^{2+} sensitivity of the BK channel may thus be estimated from changes in $V_{1/2}$ for each change in $[Ca^{2+}]_i$.[14] No decrease in "apparent" Ca^{2+} sensitivity could be seen in vasospastic myocytes using this method in different $[Ca^{2+}]_i$. Whole-cell experiments exploring the Ca^{2+} sensitivity of the BK channel were limited by smooth muscle cell contraction when $[Ca^{2+}]_i$ exceeded 500 nmol/L.

Kv Channels

Strong intracellular Ca^{2+} buffering and external Ca^{2+}-channel blocking were used to record Kv current in the absence of contaminating BK current. Kv current was of the "delayed-rectifier" type, activating and inactivating slowly, with no transient A-type rapidly inactivating component. Steady-state GV relationships for Kv current were derived from normalized tail current amplitudes obtained at –20 mV. Kv current density and membrane conductance were significantly smaller in vasospastic cells (Fig. 5–2). To ensure that this was not simply due to a shift in the GV relationship after SAH, maximum control and vasospastic slope conductances[15] were compared between +20 and + 40 mV (where channel activation was near complete). The resulting conductance density of I_{Kv} (pS/pF) was nearly halved in vasospastic myocytes.

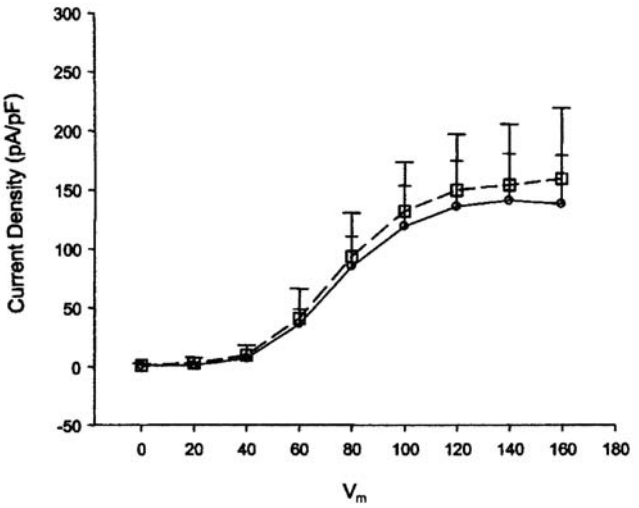


FIGURE 5–1 Graph showing large conductance, Ca^{2+}-activated K^+ (BK) currents in smooth muscle cells isolated from control dog basilar artery and from dogs 7 days after subarachnoid hemorrhage. There is no significant difference between current density, expressed as the ratio of current to capacitance, between control and vasospastic cells (n = 5–10 cells per point, values are means ± standard deviations).

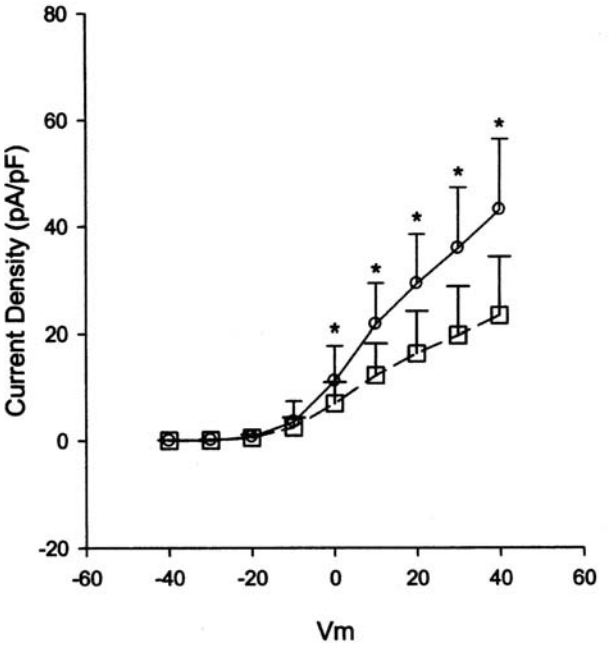


FIGURE 5–2 Graph showing voltage-gated K^+ (Kv) currents in smooth muscle cells isolated from control dog basilar artery and from dogs 7 days after subarachnoid hemorrhage. There is a significant decrease in current density, expressed as the ratio of current to capacitance, in vasospastic cells compared with control cells at more depolarized membrane potentials ($p < .05$, n = 5–10 cells per point, values are means ± standard deviations).

Discussion

We have confirmed that vasospastic dog smooth muscle cells are depolarized compared with normal basilar smooth muscle cells.[4-5] Experiments have begun to be conducted in an attempt to define the electrophysiological basis for this. BK channels regulate arterial smooth muscle membrane potential and tone by opening in response to either or both depolarization and increased $[Ca^{2+}]_i$ and thus hyperpolarizing the membrane. Decreased BK channel function diminishes the

negative feedback this pathway provides and results in increased cerebrovascular myogenic tone. BK dysfunction after SAH could therefore contribute to cerebral vasospasm. The present results, however, show that the voltage-dependence and Ca^{2+} sensitivity of the BK channel in control and vasospastic myocytes are similar. This suggests that major alterations in function of the BK channel do not occur after SAH. The role of BK channels in vasospasm, however, should be further defined using single-channel patch-clamp recordings and pharmacological methods to completely characterize the BK channel.

The second major K+ channel in cerebrovascular smooth muscle is the Kv channel. Kv channels play an important role in the regulation of membrane potential and diameter of cerebral arteries, with recent data suggesting they are the first inhibitory mechanism triggered by vascular smooth muscle depolarization.[16] Kv current density was found to be nearly halved in vasospastic myocytes compared with normal, control cells. There are many different classes of Kv channels. The specific subtype(s) of Kv channels that account for this alteration in current density await determination. They may well differ between dogs and humans. Some preliminary data presented elsewhere in this monograph suggest that the basis is a decrease in Kv channel protein, although a functional change in the channel in an absence of alteration in the actual number of channels in the membrane has not been ruled out.

Conclusion

The decrease in Kv current found in vasospastic myocytes may account for the depolarization of these cells that is noted when they are isolated from vasospastic dog basilar artery. This depolarization is consistent with the vasoconstriction observed after SAH. Additional experiments are required to determine whether the alteration in Kv current is a cause of vasospasm or a secondary result of it. An important but complex consequence of the hypothesis that Kv channels and membrane potential contribute to vasospasm is that the dihydropyridine, L-type Ca^{2+}-channel blockers would be expected to be effective antivasospastic agents. Oral and intravenous dihydropyridine Ca^{2+}-channel blockers are used clinically as prophylaxis against ischemia due to vasospasm, although their direct effects on arterial diameter are somewhat limited. This could be due to poor drug delivery across the blood–brain barrier and to systemic hypotension associated with administration of higher systemic doses rather than an actual failure of adequate doses of these drugs to affect spasm. More recently, a prolonged-release dihydropyridine Ca^{2+}-channel antagonist has been formulated in pellet form for intracranial placement close to arteries that are encased in or adjacent to thick blood clots (and which consequentially have a high risk of vasospasm). First tested successfully in a canine SAH model,[17] this has now been shown to be highly effective at preventing vasospasm in humans undergoing surgery for clipping of ruptured aneurysms, although it is less effective at distances away from the pellet perhaps due to the high lipophilicity of the drug or to lower concentrations that develop at greater distances due to diffusion.[18] These findings are interesting in view of the preceding comments about the interaction of K+ channels, membrane potential, and L-type Ca^{2+} channels.

Acknowledgment

This work was supported by grants (to R.L.M.) from the American Heart Association, National Institutes of Health (NS25946), and Brain Research Foundation. B.S.J. was supported by the Canadian Institutes of Health Research and the American Association of Neurological Surgeons.

REFERENCES

1. Harder DR. Pressure-dependent membrane depolarization in cat middle cerebral artery. Circ Res 1984;55:197–202
2. Knot HJ, Nelson MT. Regulation of arterial diameter and wall [Ca^{2+}] in cerebral arteries of rat by membrane potential and intravascular pressure. J Physiol 1998;508:199–209
3. Nelson MT, Quayle JM. Physiological roles and properties of potassium channels in arterial smooth muscle. Am J Physiol 1995;268:C799–C822
4. Waters A, Harder DR. Altered membrane properties of cerebral vascular smooth muscle following subarachnoid hemorrhage: an electrophysiological study, I: Changes in resting membrane potential (Em) and effect on the electrogenic pump potential contribution to Em. Stroke 1985;16:990–997
5. Harder DR, Dernbach P, Waters A. Possible cellular mechanism for cerebral vasospasm after experimental subarachnoid hemorrhage in the dog. J Clin Invest 1987;80:875–880
6. Zuccarello M, Bonasso CL, Lewis AI, Sperelakis N, Rapoport RM. Relaxation of subarachnoid hemorrhage–induced spasm of rabbit basilar artery by the K+ channel activator cromakalim. Stroke 1996;27:311–316
7. Macdonald RL, Zhang J, Sima B, Johns L. Papaverine-sensitive vasospasm and arterial contractility and compliance after subarachnoid hemorrhage in dogs. Neurosurgery 1995;37:962–967
8. Hamill OP, Marty A, Neher E, Sakmann B, Sigworth FJ. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pflugers Arch 1981;391:85–100
9. Catacuzzeno L, Pisconti DA, Harper AA, Petris A, Franciolini F. Characterization of the large-conductance Ca-activated K channel in myocytes of rat saphenous artery. Pflugers Arch 2000;441:208–218
10. Knaus HG, McManus OB, Lee SH, et al. Tremorgenic indole alkaloids potently inhibit smooth muscle high-conductance calcium-activated potassium channels. Biochemistry 1994;33:5819–5828
11. Cox DH, Cui J, Aldrich RW. Allosteric gating of a large conductance Ca-activated K+ channel. J Gen Physiol 1997;110:257–281

12. Cox DH, Cui J, Aldrich RW. Separation of gating properties from permeation and block in mslo large conductance Ca-activated K+ channels. J Gen Physiol 1997;109:633–646
13. Horrigan FT, Aldrich RW. Coupling between voltage sensor activation, Ca^{2+} binding and channel opening in large conductance (BK) potassium channels. J Gen Physiol 2002;120:267–305
14. Magleby KL. Gating mechanism of BK (Slo1) channels: so near, yet so far. J Gen Physiol 2003;121:81–96
15. Smirnov SV, Robertson TP, Ward JP, Aaronson PI. Chronic hypoxia is associated with reduced delayed rectifier K+ current in rat pulmonary artery muscle cells. Am J Physiol 1994;266:H365–H370
16. Cheong A, Quinn K, Dedman AM, Beech DJ. Activation thresholds of K(V), BK and Cl(Ca) channels in smooth muscle cells in pial precapillary arterioles. J Vasc Res 2002;39:122–130

6

Sphingosine-1-Phosphate–Induced Arterial Contraction and Ca^{2+} Sensitization

MASAHIKO TOSAKA, M.D., PH.D., NOBUHITO SAITO, M.D., PH.D., YASUHIRO HASHIBA, M.D., TATSUYA SHIMIZU, M.D., HIDEAKI IMAI, M.D., PH.D., TOMIO SASAKI, M.D., PH.D.

Abstract

Sphingosine-1-phosphate (S1P) is a platelet-derived bioactive lipid that induces various biological responses including vasoconstriction. The effect of S1P on vasoconstriction of the canine basilar artery was investigated in vitro, and the mechanism was examined by assessing changes in intracellular calcium ($[Ca^{2+}]_i$) and myosin light chain (MLC) phosphorylation during the contraction. Isometric tension was measured in canine basilar arterial rings from dogs. Arterial strips were loaded with fura 2-AM for simultaneous measurement of isometric tension and $[Ca^{2+}]_i$. MLC phosphorylation levels were measured by glycerol gel electrophoresis followed by western blotting. S1P induced dose-dependent contraction of the basilar artery in vitro, which was significantly inhibited by Y-27632, a highly selective Rho kinase inhibitor. S1P (10 μmol/L) caused only a slight increase in $[Ca^{2+}]_i$, although significant contraction was observed. The phosphorylation level of MLC was increased in the contracted arteries, and this increase was inhibited by Y-27632. S1P-induced contraction of canine basilar arteries may be regulated by both $[Ca^{2+}]_i$ and the Ca^{2+} sensitivity of the contractile elements. S1P-induced contraction and myosin phosphorylation may be linked to a Y-27632 sensitive pathway. Y-27632 inhibition of contraction suggests that the contraction is mediated by Rho kinase–induced Ca^{2+} sensitization mechanisms. We hypothesize that S1P may be involved in the pathogenesis of cerebral vasospasm.

Cerebral vasospasm after subarachnoid hemorrhage (SAH) is inhibited by fasudil hydrochloride (AT877) in experimental animals and clinical studies of human SAH.[1,2] Fasudil hydrochloride is a Rho kinase inhibitor that also inhibits other kinases, although it is most potent against Rho kinase. Activated Rho kinase phosphorylates myosin light chain (MLC) phosphatase, rendering it unable to dephosphorylate MLC and relax vascular smooth muscle. Fasudil relaxes smooth muscle by inhibiting Rho kinase, which leads to disinhibition of MLC phosphatase, dephosphorylation of MLC, and relaxation. The effect is not mediated by inhibition of MLC kinase.[1] In the canine double hemorrhage model of SAH, vasospasm of the basilar artery on day 7 was associated with increased phosphorylation of MLC kinase and with activation

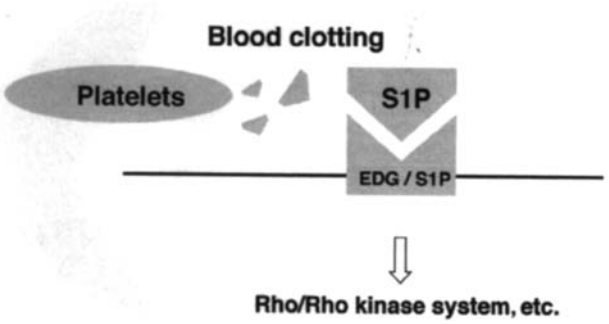


FIGURE 6–1 Sphingosine-1-phosphate (S1P) released from platelets during clot formation may activate the Rho/Rho kinase system by interaction with G-protein-coupled receptors called endothelial differentiation genes (EDG). S1P also may interact with other receptors to mediate intracellular responses.

of Rho kinase. The latter was marked by an increased phosphorylation rate of the myosin-binding subunit.[2] These data suggest that the Rho/Rho kinase intracellular signal transduction system may be important in the pathogenesis of cerebral vasospasm.

The platelet-derived bioactive lipid, sphingosine-1-phosphate (S1P), may be an upstream activator of Rho/Rho-associated kinase (Fig. 6–l).[3–6] S1P is released from platelets during blood clotting. It stimulates several intracellular signaling events, including the Rho/Rho kinase system. S1P stimulates signaling events through a family of G-protein-coupled receptors called endothelial differentiation genes (EDGs), although S1P also stimulates cells through intracellular targets in some cases. S1P has high affinity for several EDG receptors (S1P1/EDG-1, S1P2/EDG-5, S1P3/EDG-3, S1P4/EDG-6, S1P5/EDG-8).[5–8] This study investigated the effect of S1P on vasoconstriction of the canine basilar artery in vitro and determined the mechanism of this contraction by assessing changes in $[Ca^{2+}]_i$ and MLC phosphorylation during the contraction.

Materials and Methods

All experiments were performed according to the Rules Governing Animal Experimentation and the Guidelines for the Care and Use of Laboratory Animals of Gunma University School of Medicine. Rings of canine basilar artery were obtained from mongrel dogs of both sexes (8 to 12 kg), and placed in N-[2-hydroxyethyl]piperazine-N'[2-ethanesulfonic acid]-buffered Krebs solution (pH 7.4). The endothelium was chemically denuded.[9] The 4 mm long rings were placed in organ baths containing 5 mL normal Krebs–Ringer bicarbonate solution that was aerated with 95% air/5% CO_2 (pH 7.4) and maintained at 37°C using a thermocirculator. The rings were placed under 1.0 g resting tension between a hook and an isometric force transducer.[10]

Canine basilar arterial strips without endothelium were also prepared and loaded with 10 µmol/L fura 2-AM at room temperature (22–24°C). After loading, the muscle strips were washed and then placed under 1.0 g resting tension between two stainless steel hooks. One hook was anchored, and the other was connected to a strain gauge transducer to measure isometric force. The fura 2-AM fluorescence signal and isometric tension were continuously monitored. Fluorescence measurements were performed with a dual-wavelength spectrofluorometer at excitation wavelengths of 340 and 380 nm.

The extent of MLC phosphorylation in the arterial strips was determined using the urea-glycerol gel electrophoresis technique, followed by western blotting with a specific mouse monoclonal anti-MLC antibody

Results

S1P induced a dose-dependent contraction in the basilar arterial rings without endothelium (Fig. 6–2). To determine whether the Rho/Rho kinase system is involved in the S1P-induced contraction, the rings were preincubated for 15 minutes with the Rho kinase inhibitor, Y-27632 (10 µmol/L), and then cumulative amounts of S1P were added to the incubation medium. The contractile effect was significantly inhibited by Y-27632 (see Fig. 6–2). Representative tracings of the change in fluorescence signal of fura-2

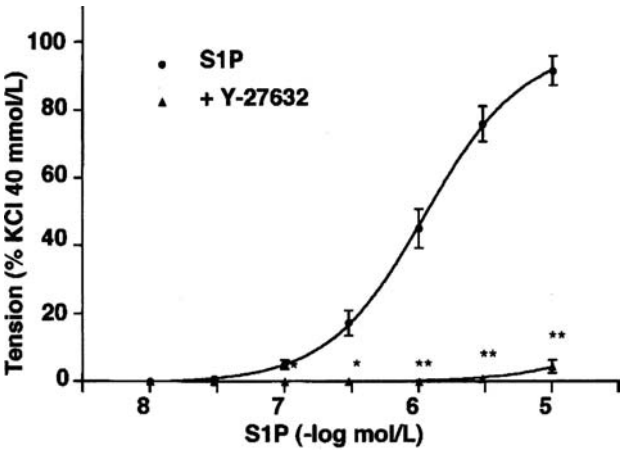


FIGURE 6–2 Graph showing that sphingosine-1-phosphate (S1P) induces contraction of isolated canine basilar artery rings. Preincubation with Y-27632 (10 µmol/L) significantly inhibited contraction to S1P. Contractions are expressed as percents of the response induced by 40 mmol/L KCl [values are mean ± standard error of the mean, n = 5 (presence of Y-27632) to 13 (absence of Y-27632)].

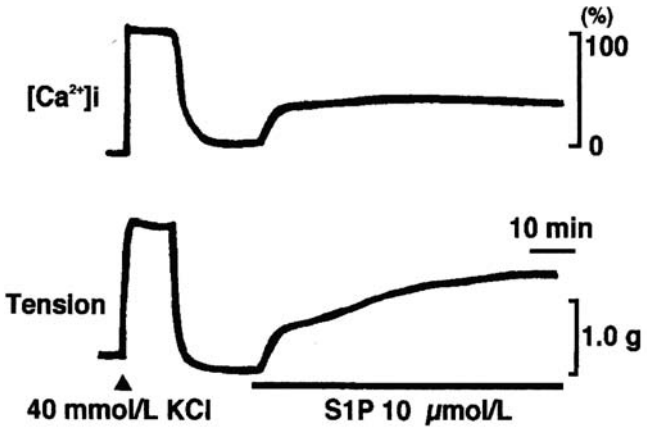


FIGURE 6–3 Typical tracings of the changes induced by sphingosine-1 -phosphate in the fura-2 fluorescence ratio ($[Ca^{2+}]_i$) (upper trace) and tension development (lower trace). The responses in the fluorescence ratio and tension to depolarization with KCl (40 mmol/L) were recorded before each experiment as a control (100%). Vertical bar in the lower trace denotes tension (g), and horizontal bar denotes time (minutes, min).

(F340/F380 ratio) and the tension induced by S1P (10 μmol/L) show that S1P caused a slight but sustained increase in $[Ca^{2+}]_i$ (Fig. 6–3). S1P-induced contraction was initially rapid and small, but subsequently became significant. The phosphorylation level of MLC was increased in the arteries contracted by S1P, but the increase was inhibited by Y-27632 (data not shown).

Discussion

Blood clotting surrounding the cerebral vessels after SAH may be an important process in terms of stopping bleeding and healing the rent in the aneurysm but may also contribute to cerebral vasospasm. Platelets isolated from the blood cause cerebral vasospasm in experimental animal models.[11] S1P is abundant in platelets and is released by stimulation with thrombin or phorbol ester.[7] Therefore, S1P released from clots formed in the subarachnoid space may be involved in vasospasm after SAH. Local production of S1P by activated platelets may be sufficient to evoke a vasocontraction of the cerebral arteries.[3]

S1P exerts a potent vasocontractile effect on the cerebral arteries but not on the peripheral arteries.[5,6] Subtype-specific S1P receptor antibodies revealed that the expression of S1P3/EDG-3 and S1P2/EDG-5 receptors is fourfold higher in the cerebral arteries compared with the aorta, whereas S1P1/EDG-1 receptor expression was similar in both types of artery. Therefore, the vasocontractile ability of S1P specific to cerebral arteries may be related to the expression of S1P receptor subtypes.[5] This difference of expression of S1P receptor subtype was thought to be related to pathogenesis of cerebral vasospasm and to be a potential target for the treatment of vasospasm.[5,6]

Tamama et al reported that S1P increases $[Ca^{2+}]_i$ in smooth muscle cells when they are in a synthetic phase.[8] In addition, S1P activated the Rho/Rho kinase pathway in these cells. These actions may combine to induce persistent vasocontraction.[3,12] The present study confirmed in intact smooth muscle cells in the arterial wall, which would be generally in a nonsynthetic phase, that S1P induced a small $[Ca^{2+}]_i$ increase associated with augmentation of the contraction. S1P-induced contraction was associated with increased phosphorylation levels of MLC, and this increase in phosphorylation was inhibited by preincubation with Y-27632, a Rho kinase inhibitor.

These preliminary results indicate that a small increase in $[Ca^{2+}]_i$ and subsequent MLC kinase activation lead to the phosphorylation of the MLC, an initial process for the contraction of smooth muscle cells. Rho kinase causes the inhibition of myosin phosphatase by phosphorylating the myosin-binding subunit of the enzyme. Modification of the enzyme system may maintain or increase the phosphorylation level of the MLC, resulting in an increase in the sensitivity of Ca^{2+} for contraction. These events may combine to induce persistent vasoconstriction of the smooth muscle cells in the cerebral arteries. S1P may induce the increase in $[Ca^{2+}]_i$ and activation of the Rho/Rho kinase system through several differential membrane receptor EDG/S1P subtypes.[5,6] Therefore, S1P may increase Rho/Rho kinase–related Ca^{2+} sensitization, resulting in contraction of the cerebral arteries and induction of cerebral vasospasm after SAH.

REFERENCES

1. Nakamura K, Nishimura J, Hirano K, Ibayashi S, Fujishima M, Kanaide H. Hydroxyfasudil, an active metabolite of fasudil hydrochloride, relaxes the rabbit basilar artery by disinhibition of myosin light chain phosphatase. J Cereb Blood Flow Metab 2001;21:876–885
2. Sato M, Tani E, Fujikawa H, Kaibuchi K. Involvement of Rho-kinase-mediated phosphorylation of myosin light chain in enhancement of cerebral vasospasm. Circ Res 2000;87:195–200
3. Tosaka M, Okajima F, Hashiba Y, et al. Sphingosine-1 -phosphate contracts canine basilar arteries in vitro and in vivo: possible role in pathogenesis of cerebral vasospasm. Stroke 2001;32:2913–2919
4. Somlyo AV. New roads leading to Ca^{2+} sensitization. Circ Res 2002;91:83–84
5. Coussin F, Scott RH, Wise A, Nixon GF. Comparison of sphingosine-1-phosphate-induced intracellular signaling pathways in vascular smooth muscles: differential role in vasoconstriction. Circ Res 2002;91:151–157
6. Salomone S, Yoshimura S, Reuter U, et al. S1P(3) receptors mediate the potent constriction of cerebral arteries by sphingosine-1-phosphate. Eur J Pharmacol 2003;469:125–134

7. Yatomi Y, Ohmori T, Rile G, et al. Sphingosine 1-phosphate as a major bioactive lysophospholipid that is released from platelets and interacts with endothelial cells. Blood 2000;96:3431–3438
8. Tamama K, Kon J, Sato K, et al. Extracellular mechanism through the EDG family of receptors might be responsible for sphingosine-1-phosphate-induced regulation of DNA synthesis and migration of rat aortic smooth-muscle cells. Biochem J 2001;353:139–146
9. Connor HE, Feniuk W. Role of endothelium in haemoglobin-induced contraction of dog basilar artery. Eur J Pharmacol 1987;140:105–108
10. Tosaka M, Hashiba Y, Saito N, Imai H, Shimizu T, Sasaki T. Contractile responses to reactive oxygen species in the canine basilar artery in vitro: selective inhibitory effect of MCI-186, a new hydroxyl radical scavenger. Acta Neurochir (Wien) 2002; 144:1305–1310
11. White RP, Hagen AA, Morgan H, Dawson WN, Robertson JT. Experimental study on the genesis of cerebral vasospasm. Stroke 1975;6:52–57
12. Sasaki T, Kassell NF, Zuccarello M. Dependence of cerebral arterial contractions on intracellularly stored Ca^{2+}. Stroke 1986; 17:95–97

7

Potential Role of Potassium Channels in Tyrosine Kinase Inhibitor–Induced Vascular Relaxation in Rat Basilar Artery: A Patch-Clamp Study

CHUL-JIN KIM, M.D., PH.D., DONG-HAN HAN, M.D., YONG-GEUN KWAK, M.D.

Abstract

Tyrosine kinase inhibitors may be useful in the management of cerebral vasospasm. It has not been reported whether K+ channels play a role in tyrosine kinase inhibitor–induced vascular relaxation of cerebral arteries. This study was undertaken to clarify the role of K+ channels in tyrosine kinase inhibitor–induced vascular relaxation and to investigate the effect of tyrosine kinase inhibitors on outward K+ currents in freshly isolated smooth muscle cells from rat basilar artery. The isolation of rat basilar smooth muscle cells was performed by specialized techniques. The whole cell currents were recorded by whole cell patch-clamp technique in freshly isolated smooth muscle cells from rat basilar artery. In the present study, genistein (n = 10), tyrphostin A-23 (n = 10), or A-25 (n = 10), all at 30 µmol/L in the bath solution, increased the amplitude of the outward K+ current. This current was completely blocked by the large conductance calcium-activated potassium channel blocker, iberiotoxin (0.1 µmol/L), and the calcium chelator, l,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid (BAPTA), in whole cell mode. In contrast, diadzein (30 µmol/L, n = 10), an inactive analogue of genistein, did not increase the amplitude of the outward K+ current. From these results, tyrosine kinase inhibitors such as genistein, tyrphostin A-23, and tyrphostin A-25 increase the activity of large-conductance, Ca^{2+}-activated K+ channels in cerebral basilar smooth muscle cells, thereby contributing to the relaxation of cerebral artery We can suggest that K+ channels may play a role in tyrosine kinase inhibitor–induced vascular relaxation in rat basilar artery.

Tyrosine kinases have been shown to be involved in the contraction of peripheral smooth muscle either by activation of receptors or by opening of Ca^{2+} channels.[1] However, little is known of their action in cerebral arteries. It is known that K+ channels are important for setting the resting membrane potential of smooth muscle and thereby modulating the excitability of the smooth muscle. It has not been determined whether K+ channels play a role in tyrosine kinase–induced vascular relaxation of cerebral artery This study used tyrosine kinase inhibitors to elucidate the role of K+ channels in the vascular relaxation

induced by these drugs. Also studied was the effect of tyrosine kinase inhibitors on outward K+ currents in freshly isolated smooth muscle cells from rat basilar artery

Material and Methods

Female Sprague-Dawley rats were anesthetized with methoxyflurane and decapitated. The basilar arteries were removed and placed in medium containing (in mmol/L): NaCl 130, KCl 5, $CaCl_2$ 0.8, $MgCl_2$1.3, glucose 5, N-[2-hydroxyethyl]piperazine-N'[2-ethanesulfonic acid] (HEPES) 10, penicillin (100 units/mL), and streptomycin (0.1 g/L). The arteries were cut into 2.0 mm rings and incubated for 1 hour at room temperature in a medium containing 0.2 mmol/L $CaCl_2$ and collagenase (type II, 0.5 g/L), elastase (0.5 g/L), hyaluronidase (type IV-S, 0.5 g/L), and deoxyribonuclease I (0.1 g/L). The rings were washed in fresh solution containing $CaCl_2$ (0.2 mmol/L), trypsin inhibitor (0.5 g/L), and deoxyribonuclease I (0.1 g/L) and then triturated gently. Cells were voltage-clamped using the whole-cell patch-clamp technique. Electrodes were positioned using a three-dimensional vernier-type hydraulic mecromanipulator (MX-630R, Soma Scientific, Irvine, CA, USA). Seals (5–10 GO) were formed by applying gentle negative pressure. Voltage steps were applied with pulse protocols driven by a computer equipped with A-D and D-A converters (DigiData 1200, Axon Instruments, Foster City, CA, USA). Data analysis was performed using pCLAMP 5/7/1. All experiments were performed at room temperature (20–26°C). The normal bath solution for whole-cell recordings was (mmol/L): NaCl 130, KCl 5, $MgCl_2$ 1.2, $CaCl_2$ 1.8, HEPES 10, glucose 5.2 with the pH adjusted to 7.4 with NaOH. Pipettes were filled with (mmol/L): KCl 140, $MgCl_2$ 0.5, $CaCl_2$ 0.1, ethylenebis(oxonitrilo)tetraacetic acid (EGTA) 0.09, HEPES 10, glucose 10 with the pH adjusted to 7.4 with KOH.

Results

After establishment of whole-cell recording mode, control recordings were collected for ~5 minutes until the current elicited by depolarization stabilized. Tyrosine kinase inhibitors were applied into the bath solution. In control studies, the whole-cell current remained stable over 10 to 20 minutes in the absence of interventions. The addition of tyrosine kinase inhibitors significantly increased the outward K+ current at around 2 to 3 minutes after application. Addition of genistein (30 µmol/L) into the bath solution increased the amplitude of the outward K+ current remarkably ($n = 10$, $p < .05$). This current was completely blocked by the large conductance calcium-activated potassium channel blocker, iberiotoxin (0.1 µmol/L) and the calcium chelator, BAPTA, in whole cell mode. Application of diadzein (30 µmol/L), an inactive analogue of genistein, did not increase the amplitude of the outward K+ current. The effect of tyrphostin A-23 on the whole-cell K+ current was tested. Application of tyrphostin A-23 (30 µmol/L) into the bath solution increased the amplitude of the outward K+ current remarkably ($n = 10$, $p < .05$). Application of tyrphostin A-25 (30 µmol/L) into bath solution increased the amplitude of the outward K+ current partially ($n = 10$, $p < .01$).

Discussion

Signal transduction pathways associated with contraction of smooth muscle constitute a complex process involving multiple regulatory mechanisms. A rapidly growing body of evidence suggests that tyrosine kinases, enzymes that phosphorylate proteins on tyrosine residues, participate in diverse signaling pathways including regulation of receptors for neurotransmitters, initiation of mitogenic responses by certain growth factors,[2] and modulation of ß-adrenergic signal transduction in fibroblasts.[3] Tyrosine kinases have been shown to be involved in the contraction of peripheral smooth muscle either by activation of receptors or by opening of Ca^{2+} channels. However, little is known of their action in cerebral arteries.

It has been established that the etiology of vasospasm is subarachnoid blood clot and that one component of the clot that contributes to vasospasm is the erythrocyte cytosol or hemolysate.[4] We previously noted that tyrosine kinase inhibitors significantly attenuated contraction of rabbit basilar artery in response to hemolysate.[5] The mechanism for the inhibitory effect of tyrosine kinase inhibitors on erythrocyte lysate–induced contraction is not clear. Our data suggested that protein tyrosine phosphorylation/dephosphorylation is a key step of signal transduction in cerebral vascular smooth muscle. Action of erythrocyte lysate in cerebral arteries may be associated with an increase in protein tyrosine phosphorylation, which participates in the contraction of cerebral smooth muscle.

The mechanism of relaxation of vascular smooth muscle and the involvement of K+ have been discussed by many authors.[6,7] When a K+ channel opens, K+ diffuses down its electrochemical gradient, transferring positive charge out of the cell, thereby making the interior of the cell more negative and driving the membrane potential in a hyperpolarizing direction. Vascular smooth muscle possesses several classes of K+ channels, including Ca^{2+}-activated, adenosine

triphosphate–sensitive, delayed rectifier, and inward rectifier types. The Ca^{2+}-activated K+ channel in smooth muscle has a large conductance and hence is referred to as a large or big-conductance channel that is uniquely sensitive to charybdotoxin but not to procaine or strychnine. These channels are voltage-dependent, activated by increased internal calcium, and inhibited by tetraethylammonium and charybdotoxin. In addition, delayed rectifiers[8] and inward rectifiers are present.

Conclusion

Our results suggest tyrosine kinase inhibitors such as genistein, tyrphostin A-23, and tyrphostin A-25 increased K+ channel activity in cerebral basilar artery smooth muscle cells, thereby contributing to the relaxation of cerebral artery. We hypothesize that the large-conductance, Ca^{2+}-activated K+ channel is important in this effect.

REFERENCES

1. Di Salvo J, Kaplan N, Semenchuk LA. Protein tyrosine phosphorylation and regulation of intracellular calcium in smooth muscle cells. In: Barany M, ed. Biochemistry of Smooth Muscle Contraction. San Diego: Academic; 1996:283–293
2. Bilder GE, Krawiec JA, McVety K, et al. Tyrphostins inhibit PDGF-induced DNA synthesis and associated early events in smooth muscle cells. Am J Physiol 1991;260:C721–C730
3. Bushman WA, Wilson LK, Luttrell DK, Moyers JS, Parsons SJ. Overexpression of c-Src enhances beta-adrenergic–induced cAMP accumulation. Proc Natl Acad Sci USA 1990;87: 7462–7466
4. Bevan JA, Bevan RD. Arterial wall changes in chronic cerebrovasospasm: in vitro and in vivo pharmacological evidence. Annu Rev Pharmacol Toxicol 1988;28:311–329
5. Kim CJ, Kim KW, Park JW, Lee JC, Zhang JH. Role of tyrosine kinase in erythrocyte lysate-induced contraction in rabbit cerebral arteries. J Neurosurg 1998;89:289–296
6. Cook NS, Quast U. Potassium channel pharmacology. In: Cook NS, ed. Potassium Channels: Structure, Classification, Function and Therapeutic Potential. West Sussex, UK: Ellis Horwood Limited; 1990:278–299
7. Cook NS. The pharmacology of potassium channels and their therapeutic potential. Trends Pharmacol Sci 1988;9:21–28
8. Bonnet P, Rusch NJ, Harder DR. Characterization of an outward K^+ current in freshly dispersed cerebral arterial muscle cells. Pflugers Arch 1991;418:292–296

8

The Role of Calcium-Activated Potassium Channels in the Mouse Model of Chronic Cerebral Vasospasm

ROBERT L. DODD, M.D., PH.D., ATSUSHI SAITO, M.D., ROBERT BRENNER, PH.D., ANDREW J. PATTERSON, M.D., PH.D., PAK CHAN, PH.D., RICHARD ALDRICH, PH.D.

Abstract

Large-(big)-conductance Calcium-activated potassium channels (BKCa) modulate arterial tone by antagonizing vessel contraction. There is evidence that the reactive oxygen species such as peroxynitrite, which can be produced from heme oxidation of blood following subarachnoid hemorrhage (SAH), can directly inhibit BKCa channel unitary conductance. This effect may contribute to cerebral artery vasospasm. This study investigated whether the abnormal contraction of vascular smooth muscle in cerebral vasospasm results from BKCa dysfunction. Transgenic knockout mice in which the regulatory (ß1) subunit of the BKCa channel was genetically disrupted were used to evaluate BKCa channel function. The ß1 subunit modulates Ca^{2+} sensitivity, and channels without this subunit have significantly decreased open probability. BKCa ß1-knockout and littermate control mice were subjected to endovascular perforation of the left anterior cerebral artery. The mice were subsequently perfused with a carbon black and gelatin mixture to cast all vessels. Morphometric analysis of vessel diameter was then performed in vitro using light microscopy A 20% reduction in proximal middle cerebral artery diameter in wild type mice following SAH was observed. BKCa ß1-knockout mice had almost a 20% reduction in vessel diameter at baseline [83.6 ± 9.8 (m, n = 13)] as compared with wild type [97.8 ± 17.6 (m, n = 17)]. Preliminary results suggest that, following SAH, there was also a trend for an ~20% reduction in proximal middle cerebral artery diameter of BKCa ß1-knockout mice as well (p < .07, n = 9). Furthermore, no significant difference in vessel diameter reduction between wild type and knockout mice was observed following SAH (p = .5). It is concluded that BK channels contribute to normal cerebral artery myogenic tone; however, preliminary results suggest they are not involved in the molecular mechanism of chronic vasospasm. Other voltage-gated potassium channels may therefore be responsible for this observed vasoconstriction. Further experiments to characterize this role are under way.

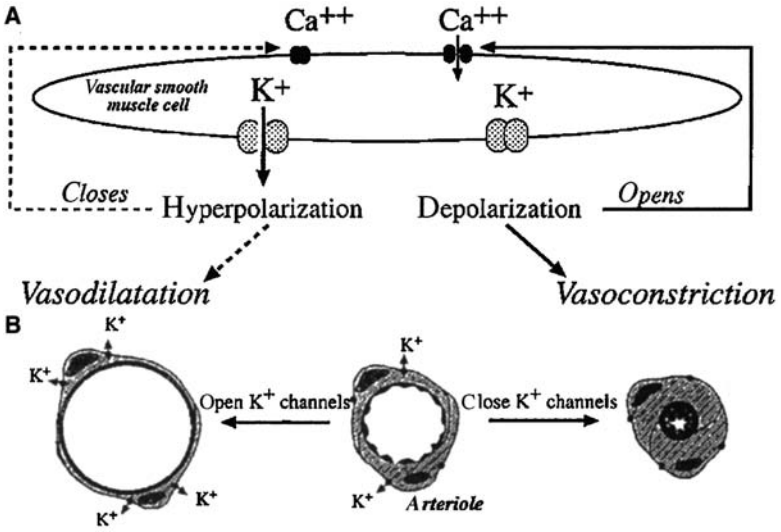


FIGURE 8–1 Schematic diagram of a vascular smooth muscle cell (A) and cross sections through an arteriole (B) showing that opening K+ channels leads to diffusion of K+ ions out of the cell, membrane hyperpolarization, closure of voltage-gated Ca^{2+} channels, and decreased intracellular Ca^{2+}, which leads to vasodilatation. Closure of K+ channels has the opposite effect (adapted from Jackson 2000[1]).

Subarachnoid hemorrhage (SAH)–induced cerebral vasospasm remains a major cause of mortality and stroke. Vasospasm is a gradually developing, prolonged constriction of the cerebral arteries following aneurysmal hemorrhage. The principal cause is blood surrounding the vessels; however, the component that induces the spasm remains unknown. No curative medical treatment currently exists. Vessel diameter is determined by vascular tone, which is regulated by ion channels in smooth muscle cell membranes.[1] The central problem in vasospasm is an abnormal contraction of vascular smooth muscle, which we hypothesize is secondary to either K+ channel dysfunction or mechanisms linked to channel activity. Ca^{2+}-activated K+ channels (BKCa) modulate arterial tone by antagonizing vessel contraction (Fig. 8–1). There is evidence that the reactive oxygen species, peroxynitrite, produced from heme oxidation of blood following subarachnoid hemorrhage (SAH) can directly inhibit BKCa channel unitary conductance and may therefore contribute to cerebral artery vasospasm. A mouse model of SAH-induced vasospasm has recently been developed[2-3] and characterized.[4-5] Transgenic knockout mice in which the regulatory (ß1) subunit of the BKCa channel was genetically disrupted have also recently been created and can be used to evaluate BKCa channel function.[6] The ß1 subunit modulates Ca^{2+} sensitivity, and channels without this subunit have significantly decreased open probability This study investigates whether the abnormal contraction of the vascular smooth muscle in cerebral vasospasm results from BKCa dysfunction.

Methods

In accordance with the guidelines of the Stanford University Animal Care and Use Committee, BKCa ß1-knockout and littermate control mice were subjected to SAH produced by endovascular perforation of the left anterior cerebral artery. Anesthesia was induced in a chamber with a mixture of 3% isoflurane, 67% N_2O, and 30% O_2. Mice were then placed in the supine position on an operating table, and the rectal temperature was maintained at 37°C by a homeothermic blanket control unit (Harvard Apparatus, Boston, MA, USA). Mice were allowed to respire spontaneously, and the anesthesia was maintained with a mixture of 1% isoflurane, 69% N_2O, and 30% O_2 during the operation.

The left common carotid artery was exposed, and the external carotid artery and its branches were isolated and coagulated. A 6–0 monofilament nylon suture, blunted at the tip, was introduced into the internal carotid artery through the stump of the external carotid artery and advanced up to the left anterior cerebral artery near the anterior communicating artery until resistance was encountered. The suture was then advanced 5 mm further to perforate the artery. It then was withdrawn immediately, allowing reperfusion and producing SAH. The incision was closed, and the mice, allowed to recover.

The animals were maintained for the next 3 days with free access to food and water. Peak vasospasm has been previously demonstrated in this model to occur 3 days after injury, with resolution after 7 days.[2–5] Therefore, at 3 days after SAH, mice were anesthetized with an intraperitoneal injection of 2,2,2-tribromoethanol, 0.2 mg/kg, and perfused through the left ventricle with heparinized saline followed by a mixture of carbon black and 10% gelatin to cast all vessels. The perfusion solution was filtered using a syringe filter, and perfusion pressure was maintained between 60 and 80 mmHg.[4] Vessel diameter was then evaluated in vitro using light microscopy.

Results

BKCa ß1-knockout and wild type control mice were bred until they were between the ages of 14 and 16 weeks. Only male animals were used to avoid potential effects of hormone-related differences in vessel diameter. Body weight prior to surgery was similar between both groups (29 ± 2 g for wild type vs. 30 ± 2 g for BKCa pi-knockout mice), as was the 15% weight reduction 3 days following SAH. BKCa ß1-knockout mice had smaller arterial diameters as compared with those from wild type mice (Fig. 8–2), with an ~13% (midbasilar), 15% (middle cerebral artery), and 19% (anterior cerebral artery) reduction in arterial diameter. This was consistent with an increase in the mean arterial blood pressure prior to surgery [114 ± 6 mmHg for wild type (n = 6) compared with 134 ± 5 mmHg for BKCa ß1-knockout animals (n = 6), p = .029].

A decrease in vascular diameter was observed after SAH in the vessels surrounded by the SAH. Previous experiments demonstrated that the maximum reduction occurred in the proximal middle cerebral artery secondary to this anatomy being the most likely site of arterial puncture.[2,3] SAH caused a reduction in arterial diameter that varied with the extent and size of hemorrhage. If the BK channel is a molecular target for free radical–mediated vasoconstriction, genetically disabling this channel should result in no observable

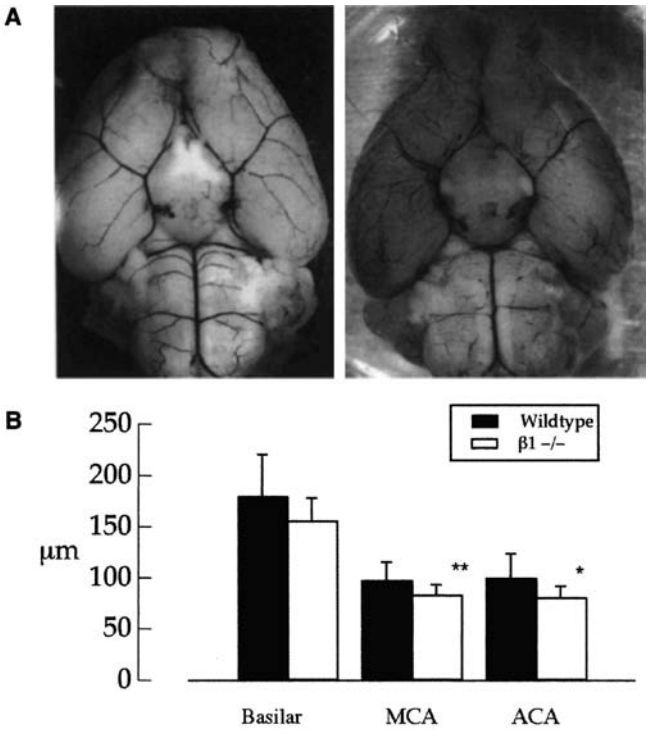


FIGURE 8–2 (A) Light micrographs of ventral surfaces of wild type C57BL6 (left) and BKCa ß1-knockout (right) mouse brains illustrating typical vascular anatomy observed following perfusion casting with a carbon black/gelatin mixture. (B) Bar histograms of the mean (± SEM) arterial diameters of the midbasilar, proximal middle cerebral (MCA), and proximal anterior cerebral arteries (ACA) from wild type (filled bars) and BKCa ß1-knockout (open bars) mice. The values for wild type mouse vessels were 180 ± 41 (n = 7), 97.8 ± 17.6 (n = 17), and 99.9 ± 23.9 µm (n = 9), respectively. Corresponding diameters for the BKCa ß1-knockout mouse vessels were 156 ± 22 (n = 12), 83.6 ± 9.8 (n = 13), and 81 ± 10.7 µm (n = 12). The middle and anterior cerebral arteries were significantly smaller in knockout compared with wild type mice (** p < .01 and * p < .08), whereas there was a statistically insignificant trend for the midbasilar artery to be larger in wild type compared with knockout mouse arteries (p = .13).

A decrease in vascular diameter was observed after SAH in the vessels surrounded by the SAH. Previous experiments demonstrated that the maximum reduction occurred in the proximal middle cerebral artery secondary to this anatomy being the most likely site of arterial puncture.[2,3] SAH caused a reduction in arterial diameter that varied with the extent and size of hemorrhage. If the BK channel is a molecular target for free radical–mediated vasoconstriction, genetically disabling this channel should result in no observable

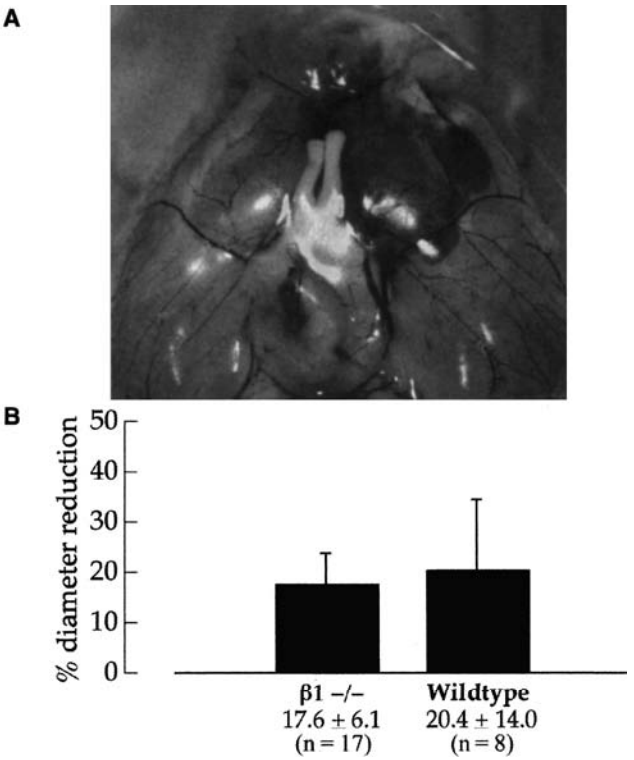


FIGURE 8–3 (A) Light micrograph of the ventral surface of the brain from a BKCa ß1-knockout mouse removed 3 days following subarachnoid hemorrhage (SAH) induced via the perforation of the left middle cerebral–anterior cerebral bifurcation. Diffuse hemosiderin staining of the subarachnoid spaces and underlying parenchyma is evident, as is the reduction in the middle cerebral artery (MCA) diameter secondary to cerebral vasospasm. (B) Bar graph demonstrating mean (± SEM) reduction in proximal MCA diameter of vasospastic vessels as compared with the vessels on the uninjured side. The values for the percent reduction in vessel diameters are 17.6 ± 6 (n = 8) and 20.4 ± 14 µm (n = 17) for the wild type and knockout vessels, respectively. No significant difference was observed in the values between these two groups (p = .5).

vasospasm following SAH. If the BK channel is not the target, vasoconstriction will still occur. For hemorrhages of similar extent (grades 2 and 3 according to the scale of Parra et al[4]), a similar reduction of vessel diameter between wild type and BKCa ß1-knockout vessels was observed (wild type 22 ± 7 µm, n = 8; BKCa ß1-knockout 21 ± 15 µm, n = 17; p = .5, Fig. 8–3). In control animals and those in which there was no visible SAH, no reduction in arterial diameter was evident.

Discussion

Although it is clear that K+ channel conductances have an important function in controlling cerebral artery tone (and therefore diameter) and that BK channels, which are the most predominant K+ channels in cerebral vascular smooth muscle, are critical in modulating this vascular tone, preliminary results suggest BK channels are not the target for chronic cerebral vasospasm. Recent electrophysiological data from enzymatically isolated canine myocytes supports this finding.[7] Aihara et al have demonstrated no significant change in BK channel current density, activation kinetics, single-channel conductance, and Ca^{2+} sensitivity in canine myocytes from animals that were rendered vasospastic using the standard double-hemorrhage model as compared with those used as controls.[7] Therefore, two different models have provided results that support the same conclusion.

This study is limited in its lack of confirmation that other important proteins known to be involved in arterial smooth muscle contraction are not upregulated in the knockout mice. In particular, it is unclear if functional disabling of BKCa channels results in upregulation of other transmembrane conductances that affect the membrane potential and therefore vessel diameter. A second limitation of this study is that despite electrophysiological evidence of a lack of BKCa channel function in cerebral myocytes from the BKCa ß1-knockout mice,[6] the electrophysiological effects of SAH-induced injury on these cells was not examined directly.

There are four types of K+ channels known to be present in vascular smooth muscle [BKCa, voltage-gated or delayed rectifier (K_v), adenosine triphosphate–sensitive, and inwardly rectifying (K_{ir}) channels]. Any of these, including various subtypes within each class, could in theory bring about a change in the membrane potential that would result in hyperpolarization and vasorelaxation. Although this study does not evaluate the role of K+ channels other than the BKCa channel, perhaps one of the other types of K+ channels is the effector of free-radical mediated injury that occurs in cerebral vasospasm. There is evidence in the literature derived from both pharmacological and electrophysiological experiments that implicates K_v channels in this role.[7] The complex nature of cellular signal transduction mechanisms makes their study difficult. The murine model, however, has substantial advantages, particularly the ability to genetically manipulate the animals, and the investigators believe that it will surely serve as an important method of investigating cerebral vasospasm.

REFERENCES

1. Jackson WF. Ion channels and vascular tone. Hypertension 2000;35:173–178
2. Kamii H, Kato I, Kinouchi H, et al. Amelioration of vasospasm after subarachnoid hemorrhage in transgenic mice overexpressing CuZn-superoxide dismutase. Stroke 1999;30:867–871
3. Saito A, Kamii H, Kato I, et al. Transgenic CuZn-superoxide dismutase inhibits NO synthase induction in experimental subarachnoid hemorrhage. Stroke 2001;32:1652–1657
4. Parra A, McGrit M, Sheng H, et al. Mouse model of subarachnoid hemorrhage associated vasospasm: methodological analysis. Neurol Res 2002;24:510–515
5. Lin C, Calisaneller T, Ukita N, et al. A murine model of subarachnoid hemorrhage-induced vasospasm. J Neurosci Methods 2003;123:89–97
6. Brenner R, Perez GJ, Bonev AD, et al. Vasoregulation by the betal subunit of the calcium-activated potassium channel. Nature 2000;407:870–876
7. Aihara Y, Jahromi BS, Yassari R, Nikitina E, Agbaje-Williams M, Macdonald RL. Molecular profile of vascular ion channels after experimental suabarachnoid hemorrhage. J Cereb Blood Flow Metab. 2004;24:75–83

9

Protein Kinase C Isoforms, Rho Kinase, and Myosin Light Chain Phosphorylation as Mechanisms of Cerebral Vasospasm After Subarachnoid Hemorrhage

SHIGERU NISHIZAWA, M.D., PH.D., MASAYO KOIDE, M.S., KAZUO OBARA, PH.D., KOICHI NAKAYAMA, PH.D., MITSUO YAMAGUCHI, M.D.

We have detected four protein kinase C (PKC) isoforms in canine basilar arteries.[1–3] Among them, the PKC d isoform translocated to the membrane fraction from the cytosol fraction in the earlier phase of vasospasm, and PKC a translocated in the later phase in the two-hemorrhage canine model of subarachnoid hemorrhage (SAH).[1–3] To confirm this finding, we conducted a subsequent experiment in which we treated dogs by injection of PKC inhibitors into the cisterna magna. Rottelein, a specific PKC d inhibitor,[4] inhibited the translocation of PKC d and the earlier phase of vasospasm but not PKC a translocation or the later phase of vasospasm. Chelerythrine, a classical/novel PKC inhibitor,[5] inhibited the translocation of both PKC d and a and the entire course of vasospasm, especially the later phase.[3] These results indicated that PKC d is involved in the initiation of vasospasm after SAH, and PKC a in its maintenance.

Phosphorylation of the myosin light chain (MLC_{20}) has been reported to play a particularly important role in the initial phases of smooth muscle contraction, although the MLC_{20} phosphorylation level declines even when vascular contraction is sustained.[6] The role of MLC_{20} phosphorylation in contributing to sustained vascular contraction has recently been reevaluated from a viewpoint of Ca^{2+} sensitization.[7–10] A low molecular G-protein, Rho, and its effecter, Rho kinase, have been reported to contribute to Ca^{2+} sensitization, resulting in sustained MLC_{20} phosphorylation and long-lasting vascular contraction.[7–10]

Vasospasm after SAH is pathologically prolonged contraction of the cerebral arteries that persists for a few weeks after SAH. It has, however, not been determined whether a sustained high phosphorylation level of MLC_{20} contributes to the pathophysiological mechanism of vasospasm or how Rho/Rho kinase and PKC isoforms interact with each other during the vasospasm. This experiment investigated the role of MLC_{20} phosphorylation, especially from a viewpoint of Rho/Rho kinase, in the mechanism of vasospasm, and identified the relation between the roles of PKC isoforms and Rho/Rho kinase in an experimental therapy study using Y-27632, a specific inhibitor of Rho kinase.[8]

Materials and Methods

Control Study and Experimental Therapy Study

A modified two-hemorrhage canine model[11] was used for SAH and vasospasm. In the modified two-hemorrhage model, autologous blood was injected on days 1 and 4 to more accurately mimic the time course of vasospasm in humans. The time course and the extent of vasospasm in the modified and the original models were not significantly different.[2-3]

Fifteen beagle dogs were randomly divided into two groups: a control group (n = 9) and an experimental therapy group (Y-27632 group, n = 6). The control group consisted of pharmacologically untreated animals that were sacrificed on day 4 before the second autologous

blood injection (n = 3), 1 hour after the second injection on day 4 (n = 3), or on day 7 (n = 3). In the Y-27632 group, all six dogs were treated with Y-27632, a specific inhibitor of Rho kinase,[8] to assess its effect on angiographic vasospasm, translocation of PKC d and a, and the MLC_{20} phosphorylation level. A 3 mL volume of sterile phosphate-buffered saline containing 10 µmol/L of Y-27632 was injected into the cisterna magna on day 4, just before the second injection of blood and daily thereafter until day 7. Dogs were sacrificed on day 4 after the second injection (n = 3) or on day 7 (n = 3). After sacrifice, the basilar arteries of both the control group and the Y-27632 group were excised as soon as possible, dissected under a microscope, and prepared for western blotting to examine them for translocation of PKC d and a isoforms and for MLC_{20} phosphorylation.

Angiography

Angiography was performed as described elsewhere.[1–3] After obtaining a control image, 3 mL of autologous blood was manually injected into the cisterna magna, with the head of the dog maintained downward for 10 minutes so that blood would collect around the basilar artery (day 1). Angiography was repeated on day 4, before the second injection of blood, 1 hour after the second injection, and on day 7. The diameter of the basilar artery (in millimeters) was measured as described elsewhere,[1–3] and diameter was expressed as a percentage of diameter on the control angiogram on day 1 (control = 100%).

Translocation of PKC Isoforms and MLC_{20} Phosphorylation by Western Blot

The basilar arteries of the dogs sacrificed at the times indicated earlier were removed, the endothelia were denuded, and the samples were prepared as described previously.[1–3,12] Translocation of PKC isoforms was investigated by Western blot analysis using the method already described.[1–3] The amount of PKC was quantified by densitometric scanning of immunostained nitrocellulose blots. The intracellular distribution of PKC was then expressed as a percent of the total amount of each PKC isoform. The level of MLC_{20} phosphorylation was quantified using a modification of the method previously described.[3-13] The extent of MLC_{20} phosphorylation was quantified with densitometric scanning of immunostained nitrocellulose blots and expressed as moles of phosphate/moles of MLC_{20}.

Statistical Analysis

All data are expressed as means ± standard error of the mean (SEM). Paired or unpaired Student's t-tests were used to test for statistical differences between two means. The statistical significance of differences between groups was established according to Dunnett's multiple comparison test after an analysis of variance (ANOVA). P values of < .05 were considered to be significant.

Results

Angiography

There were no statistically significant differences between the control group and Y-27632 group in arterial diameters on day 4 before the second injection or on day 7. The difference in diameter on day 4 after the second injection, however, was statistically significantly different between the two groups (Fig. 9–1, $p < .01$).

Changes in Translocation of PKC Isoforms

The PKC d values in the cytosol fraction in the control group on day 4 after the second injection (n = 3) and on day 7 (n = 3) were significantly decreased compared with the normal intact canine basilar arteries (baseline control, n = 3) and the values on day 4 before the second injection (n = 3, $p < .01$, Table 9–1). The PKC d values in the membrane fraction on day 4 after the second injection (n = 3) and on day 7 (n = 3) were significantly higher than those in the normal

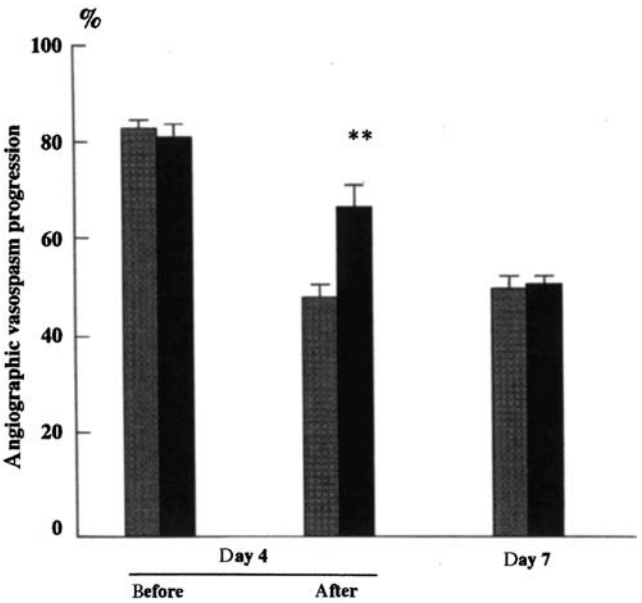


FIGURE 9–1 Bar graph showing the results of the progression of angiographic vasospasm in the control group and Y-27632 group. Shaded bars are control group, and solid bars are Y-27632 group (values are means ± SEM, $**p < .01$ compared with the control group).

TABLE 9–1 Effect of Y-27632 on Intracellular Distribution of PKC d.*

Group	Baseline Control	Day 4		Day 7
		Before Injection	After Injection	
Control				
Cytosol	66.2 ± 4.3	65.8 ± 5.7	30.2 ± 4.9	33.1 ± 5.3
Membrane	33.8 ± 4.3	34.2 ± 5.7	69.8 ± 4.9	66.9 ± 5.3
Y-27632				
Control			69.1 ± 5.1†	30.7 ± 6.4
Membrane			30.7 ± 5.1†	69.3 ± 6.4

* Values are means ± SEM.
† $p < .01$ compared with control values.

intact arteries (baseline control, $n = 3$) and the values on day 4 before the second injection ($n = 3$, $p < .01$). In the Y-27632 group, the PKC d value in the cytosol fraction on day 4 after the second injection was significantly higher than on the same day in the control group, and the value in the membrane fraction was significantly decreased compared with the control group ($n = 3$, $p < .01$). On the other hand, there were no statistically significant differences between the control group and the Y-27632 group in either the cytosol or the membrane fraction values on day 7 ($n = 3$, see Table 9–1).

Assessment of the translocation of PKC a in the control group showed that the membrane fraction value of PKC a on day 7 was significantly higher than in the normal intact artery (baseline control) and in the artery on day 4 before the second injection and after the second injection (each $n = 3$, $p < .05$, Table 9–2). In the *Y-27632* group, the intracellular distribution of PKC a on day 4 after the second injection was not significantly different from the value in the control group. On day 7, Y-27632 was not found to have any inhibitory effect on PKC a translocation to the membrane fraction.

Changes in MLC_{20} Phosphorylation

Among the control dogs, the levels of MLC_{20} phosphorylation in the normal intact artery (baseline control) were not significantly different from those on day 4 before the second injection (Fig. 9–2). However, the phosphorylation levels on day 4 after the second injection and on day 7 were significantly higher than in the normal intact artery and on day 4 before the second injection ($p < .01$). MLC_{20} phosphorylation on day 4 after the second injection ($n = 3$) and on day 7 ($n = 3$) were not significantly different. In the Y-27632 group, MLC_{20} phosphorylation was significantly reduced on day 4 after the second injection ($p < .01$) and on day 7 ($p < .05$) compared with control values at those times (see Fig. 9–2).

Discussion

MLC_{20} Phosphorylation, PKC Isoforms, and Vasospasm after SAH

It has been reported that prolonged MLC_{20} phosphorylation might be a major pathophysiological mechanism involved in the genesis of vasospasm after

TABLE 9–2 Effect of Y-27632 on Intracellular Distribution of PKC a*

Group	Baseline Control	Day 4		Day 7
		Before Injection	After Injection	
Control				
Cytosol	66.1 ± 3.6	60.7 ± 2.6	57.8 ± 2.5	41.1 ± 4.5
Membrane	33.9 ± 3.6	39.3 ± 2.6	42.2 ± 2.5	59.2 ± 4.5†
Y-27632				
Control			69.2 ± 6.1	27.7 ± 4.1
Membrane			30.8 ± 6.1	72.3 ± 4.1

* Values are means ± SEM.
† $p < .05$ compared with values in membrane fraction of control group at other times.

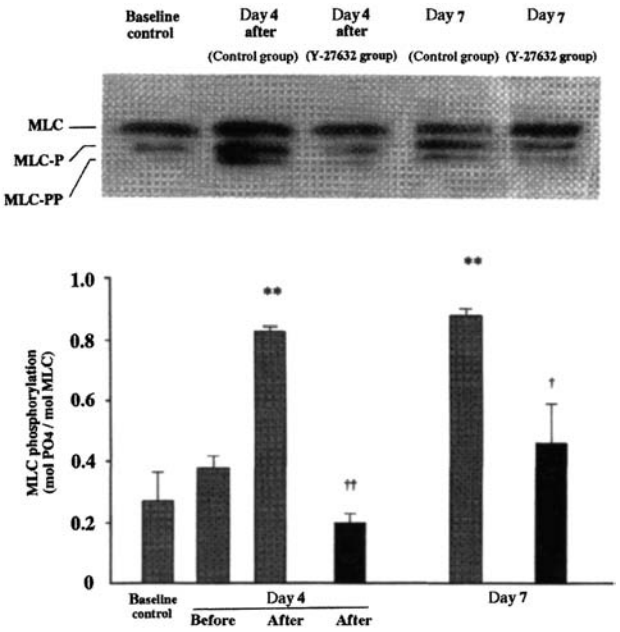


FIGURE 9–2 Myosin light chain (MLC_{20}) phosphorylation levels in the control group and Y-27632 group. Top row shows western blot of MLC_{20} phosphorylation. Only the mono- and di-phosphorylated forms of MLC_{20} were detected on all days tested in both the control and the Y-27632 groups in the present experiments. Bottom row is a bar graph showing that in the control group, phosphorylation of MLC_{20} was significantly enhanced on day 4 after the second injection, and this increase continued until day 7. In the Y-27632 group, MLC_{20} phosphorylation was significantly reduced on day 4 after the second injection and on day 7 compared with control values on those days. Shaded bars are normal intact arteries (baseline control) and control group and solid bars are Y-27632 group [values are means ± SEM, ** $p < .01$ vs. normal intact canine basilar artery (baseline control) and day 4 before the second injection, † $p < .05$ vs. the control group and †† $p < .01$ vs. the control group].

SAH.[14,15] However, it has also been reported that the increased levels of MLC_{20} phosphorylation that are seen acutely during smooth muscle contraction decline in the sustained phase of vascular contraction.[6] Thus, the role of MLC_{20} phosphorylation in the mechanism of long-lasting vascular contraction, such as vasospasm after SAH, has remained unclear. In recent studies, the role of MLC_{20} phosphorylation has been reevaluated from a viewpoint of Rho/Rho kinase.[7–10] The low molecular weight G-protein Rho and its effecter, Rho kinase, play a significant role not only in the activation of MLC kinase but also in the inhibition of MLC phosphatase. Activation of Rho kinase can lead to long-lasting phosphoryation of MLC_{20}.[7–10] Furthermore, Rho and Rho kinase have been thought to play a role in the increase in Ca^{2+} sensitization.[7–10] Based on these mechanisms, Rho/Rho kinase is considered to play a significant role in sustained vascular contraction.[7–10]

The relationship between PKC isoforms and MLC_{20} phosphorylation from a viewpoint of Rho/Rho kinase in vasospasm has not been clarified, and thus the purpose of the present experiment was to investigate the relation between the roles of the two kinase systems in the mechanism of vasospasm.

Effect of Y-27632 on Vasospasm and the Relation between the Roles of PKC Isoforms and Rho/Rho Kinase in Vasospasm

In the present experiment, Y-27632 was found to significantly inhibit the earlier phase of angiographic vasospasm, but not the later phase. A high phosphorylation level of MLC_{20} was observed on day 4 after the second injection, and this persisted until day 7 in the control group. Y-27632 significantly inhibited the high MLC_{20} phosphorylation levels both on day 4 after the second injection and on day 7 (compared with untreated SAH dogs), and it also inhibited the translocation of PKC d to the membrane fraction on day 4 after the second injection, but not on day 7. Translocation of PKC a was unaffected by Y-27632.

Results of both the previous and the present experiments clearly indicate that Rho kinase modulates the activation of PKC d and is involved in the initiation of vasospasm. The Rho/Rho kinase system may be located upstream of PKC d, and the vasospasm in the earlier phase may be induced by the activation of PKC d through Rho kinase activation. On the other hand, the maintenance of vasospasm might not be attributable to the Rho/Rho kinase system or to MLC_{20} phosphorylation. The maintenance of vasospasm may be dependent on PKC a translocation. Y-27632 had no effect on the translocation of PKC a, which indicates that PKC a and the Rho/Rho kinase systems might be functionally independent.

REFERENCES

1. Nishizawa S, Obara K, Nakayama K, et al. Protein kinase C d and a are involved in the development of vasospasm after subarachnoid hemorrhage. Eur J Pharmacol 2000;398:113–119
2. Nishizawa S, Obara K, Nakayama K, et al. Which protein kinase C isoforms are involved in the development of vasospasm after subarachnoid hemorrhage? Acta Neurochir Suppl 2001;77: 21–24
3. Nishizawa S, Obara K, Koide M, et al. Specific attenuation of canine vasospasm after subarachnoid hemorrhage by protein kinase C inhibitors despite augmented phosphorylation of myosin light chain. J Vasc Res 2003;40:169–178
4. Gschwendt M, Muller HJ, Kielbassa K, et al. Rottlerin, a novel protein kinase inhibitor. Biochem Biophys Res Commun 1994;199:93–98
5. Herbert JM, Augereau JM, Gleye J, Maffrand JP. Chelerythrine is a potent and specific inhibitor of protein kinase C. Biochem Biophys Res Commun 1990;172:993–999
6. Driska SP, Aksoy MO, Murphy RA. Myosin light chain phosphorylation associated with contraction in arterial smooth muscle. Am J Physiol 1981;240:C222–C233

7. Kitazawa T, Kobayashi S, Horiuti K, Somlyo AV, Somlyo AP. Receptor-coupled, permeabilized smooth muscle: role of the phosphatydil inositol cascade, G-protein and modulation of the contractile response to Ca^{2+}. J Biol Chem 1989;264:5339–5342
8. Uehata M, Ishizaki T, Satoh H, et al. Calcium sensitization of smooth muscle mediated by a Rho-associated protein kinase in hypertension. Nature 1997;389:990–994
9. Somlyo AP, Somlyo AV. Signal transduction by G-proteins, rho-kinase and protein phosphatase to smooth muscle and non-muscle myosin II. J Physiol 2000;522:177–185
10. Nobe K, Paul RJ. Distinct pathways of Ca^{2+} sensitization in porcine coronary artery: effects of Rho-related kinase and protein kinase C inhibition on force and intracellular Ca^{2+}. Circ Res 2001;88:1283–1290
11. Varsos VG, Liszczak TM, Han DH, et al. Delayed cerebral vasospasm is not reversible by aminophylline, nifedipine, or papaverine in a "two-hemorrhage" canine model. J Neurosurg 1983;58:11–17
12. Nakayama K. Active and passive mechanical properties of ring and spiral segments of isolated dog basilar artery assessed by electrical and pharmacological stimulations. Blood Vessels 1988;25:285–298
13. Obara K, de Lanerolle P. Isoproterenol attenuates myosin phosphorylation and contraction of tracheal muscle. J Appl Physiol 1989;66:2017–2022
14. Sato M, Tani E, Fujikawa H, Kaibuchi K. Involvement of Rho-kinase-mediated phosphorylation of myosin light chain in enhancement of cerebral vasospasm. Circ Res 2000;87:195–200
15. Butler WE, Peterson JW, Zervas NT, Morgan KG. Intracellular calcium, myosin light chain phosphorylation, and contractile force in experimental cerebral vasospasm. Neurosurgery 1996; 38:781–787

SECTION II

Remodeling and Inflammation

10

Magnesium in Subarachnoid Hemorrhage: Is It That Simple?

NIRAV SHAH, M.D. AND J. MARC SIMARD, M.D., PH.D.

Cerebral vasospasm is a major complication of subarachnoid hemorrhage (SAH), with up to a threefold increase in mortality within the first 2 weeks after the event. In survivors, delayed cerebral ischemia and infarction are the leading causes of disability due to vasospasm. Current management and clinical prevention of vasospasm after SAH consists of both nimodipine (dihydropyridine calcium channel blocker) and hypervolemic, hypertensive, hemodilutional (hemodynamic) therapy along with neuroradiological intervention, when indicated. Despite recent advances in the management of aneurysmal SAH, vasospasm remains the major cause of disability, with a 25% decrease in favorable outcome.[1] Because of this, adjunctive therapies are being developed based on the etiology of vasospasm at the cellular and molecular levels. Numerous experimental and clinical data have suggested that hypomagnesemia causes an elevation of intracellular calcium concentration and, thus, vascular smooth muscle contraction, as well as the formation of free radicals, inflammatory agents, and specific growth factors. Because magnesium is a known vasodilator of pial blood vessels, it has been tested as an adjunctive therapy to reduce the incidence of vasospasm. Successful preliminary clinical data seem to justify further study into the molecular and biochemical effects of magnesium on endothelial and smooth muscle function. Elucidating the underlying molecular basis of its metabolism in blood vessels will advance our understanding of magnesium as a novel protective agent in the reduction of the morbidity and mortality of patients suffering from SAH-induced vasospasm.

Magnesium Therapy in Vasospasm—Clinical Data

Magnesium is the one of the most abundant cations in living cells, playing a significant role in various cellular processes. Magnesium ion, having a divalent positive charge with a diameter of 1.2 A, has the highest charge density of all of the ions found in cells.[2] Consequently, magnesium binds to anionic sites such as polyphosphates [adenosine triphosphate (ATP)]. Several hundred enzymes directly or indirectly are dependent on Mg-ATP for carbohydrate, fat, protein, and electrolyte metabolism. The exact cellular mechanisms underlying the modulatory actions of magnesium on vascular smooth muscle are still debated, but the antagonistic relationship of magnesium and calcium may be important. Because it inherently shares a similar biochemical profile to that of synthetic calcium channel antagonists, magnesium is considered a physiological calcium blocker.[3] The cellular calcium concentration is a major determinant of vascular smooth muscle contractile properties. Thus, magnesium, as a blocker of intracellular calcium metabolism, has been postulated to be a useful adjunct in the prevention of cerebral vasospasm.

Several studies indicate promising salutary effects of magnesium therapy in cerebral vasospasm from SAH.[4–8] These findings are reinforced by studies showing beneficial effects in animal models.[9–11] Evidence obtained using experimental models of brain injury and cerebral ischemia indicate that magnesium may also have a role as a neuroprotective agent. $MgSO_4$ therapy has already been demonstrated to be both safe and effective in preventing neurological complications in obstetrical patients with eclampsia[8] as well as in cardiovascular diseases. Clinically, the serum magnesium levels are usually maintained within the range of 4.0 to 5.5 mg/dL throughout the treatment period. No study advocates the sole use of $MgSO_4$ therapy; instead, treatment of choice is early use of surgery or endovascular treatment, followed by either or both aggressive hemodynamic therapy and

calcium channel blockers for vasospasm prophylaxis. Veyna et al[8] measured clinical outcomes using the Glasgow outcome scale (GOS). Mean GOS scores were 3.8 and 3.6 in the treatment and control groups, respectively,[8] with a higher percentage of patients obtaining GOS scores of 4 or 5 in the group treated with $MgSO_4$. Overall, larger studies are needed to evaluate this trend further. Notably, significant adverse effects from the treatment of $MgSO_4$ did not occur in any of the studies reviewed.

Transcranial Doppler examination, although not wholly accurate, provides a reasonable assessment of vasospasm trends based on changes in mean middle cerebral artery velocities. Lower velocities indicate a vasodilatory response to therapy of otherwise spastic vessels. Further studies in the measurement of regional cerebral blood flow and oxygen consumption would help to support the reversal of vasospasm and, thus, the overall efficacy of $MgSO_4$ therapy. Improvements in cerebral perfusion may also be documented by angiogram. Chia et al[7] classified the beneficial effect of $MgSO_4$ therapy based on the need for endovascular treatment of vasospasm. Their study supported the use of $MgSO_4$ therapy in the prevention of SAH-induced vasospasm as determined by the lesser need for neuroradiological intervention.

Channel-Blocking Mechanism

Influx of extracellular calcium into vascular smooth muscle cells plays a critical role in the development of vasospasm. The sustained influx of calcium into vascular smooth muscle results in the activation of various second messenger systems that promote contractility. This influx has been postulated to occur through voltage-operated calcium channels (VOCCs), specifically L-type calcium channels.[12] Recent studies have theorized that voltage-independent calcium channels and store-operated calcium channels also permit calcium influx, which contributes to SAH-induced vasospasm. Nimodipine and nicardipine, both calcium channel blockers, have been used clinically to prevent calcium entry into vascular smooth muscle cells and thereby inhibit vasospasm. Similarly, magnesium, because of a similar biochemical profile, also may act as a calcium channel blocker. Bara and Guiet-Bara[13] demonstrated that magnesium, like nifedipine, reduced the depolarization of smooth muscle membranes. They found that effects of serotonin, which facilitates calcium influx through VOCCs, was reversed by the addition of magnesium. These data suggest that magnesium regulates calcium influx through L-type calcium channels in vascular smooth muscle. This regulation occurs because L-type calcium channels have binding sites for magnesium within the C-terminal region of the a-subunit.[3] Depletion of intracellular magnesium relieves the magnesium block. Furthermore, when calcium channels are phosphorylated by a cyclic adenosine monophosphate (cAMP)–mediated mechanism, their sensitivity to magnesium decreases, allowing influx of calcium and the subsequent activation of contractile mechanisms. The direct binding of guanosine triphosphate (GTP) in the same intracellular C-terminal side of the a-subunit also causes a block of the L-type calcium channel. cAMP-mediated phosphorylation results in unbinding of these blocking substances through conformational change of the channel protein. According to Chakraborti et al,[3] the possible physiological relevance of such substantial inhibition by magnesium and GTP is that calcium channels may be controlled over a wide dynamic range.

Signal Transduction Mechanism

The activation of cellular signaling pathways by low extracellular magnesium levels appears to play an integral role in excitation–contraction coupling. Hypomagnesemia increases both calcium uptake and calcium release from the sarcoplasmic reticulum, causing vascular smooth muscle contraction. Yang et al[14] showed that low magnesium produces a transient intracellular calcium ion peak followed by a slow, sustained, and elevated plateau of intracellular calcium concentration. Low magnesium facilitates calcium entry via the L-type calcium channel, which, in turn, triggers calcium-induced calcium release via the sarcoplasmic reticulum. This calcium-induced calcium release may activate various kinases responsible for excitation–contraction of vascular smooth muscle. More specifically, the low-magnesium-induced contractions were attenuated by tyrosine kinase and mitogen-activated protein (MAP) kinase antagonists such as Src homology 2 (SH2) inhibitor peptide. Thus, both tyrosine and MAP kinase signaling pathways may be involved in magnesium-related vascular smooth muscle contraction.

Another signal transduction-mediated effect of magnesium on vascular smooth muscle relaxation is the ß-adrenergic-G protein-cAMP system.[15] This system is the primary signaling system mediating the ionotropic physiology of the heart. Stimulation of rat heart muscle with a ß-adrenergic agonist causes a net magnesium efflux associated with a marked increase in the amplitude of contraction.[3] These data demonstrate the opposing effects of catecholamines and magnesium. cAMP may play a key role in the magnesium regulation through ß-adrenergic receptors. An increase in cAMP via the activation of ß-adrenergic receptors induces magnesium efflux.[3] Magnesium

itself affects the function of G-proteins and G-protein mediated signaling, specifically G_ia, which activates signaling pathways to blunt normal catecholamine functions such as vascular contractility and tone. This mechanism may protect against the vaso-occlusive effects of elevated catecholamines.

Growth Factuor Stimulation of Vascular Cell Proliferation

Growth factors from platelets are increased in the cerebrospinal fluid of patients with SAH.[16,17] The coagulation of subarachnoid blood activates platelets, which release potent mitogens for vascular smooth muscle platelet-derived growth factor-AB (PDGF-AB), transforming growth factor-ß$_1$ (TGFß$_1$), and vascular endothelial growth factor (VEGF). Borel et al[16] observed smooth muscle and fibroblast proliferation after SAH in a mouse model in conjunction with PDGF protein at the affected sites. Growth factor–induced endothelial proliferation in response to SAH may induce vascular stiffening, which contributes to vasospasm.[16] It has been postulated that magnesium may decrease this potent mitogen system by antagonizing its effect on intracellular calcium-induced pathways.

Oxidative Endothelial Cytotoxicity and Inflammatory Response

Numerous experimental and clinical data indicate that hypomagnesemia induces the formation of oxygen radicals and proinflammatory agents that affect endothelial cell structure and function. Studies on magnesium-deficient animals consistently show an increase in the production of lipid peroxidation-derived free radicals and subsequent tissue injury.[3] This tissue injury may simulate growth factor–induced vascular cell proliferation, which contributes to vasospasm. Further research has demonstrated that hypomagnesemia results in the chronic toxicity of iron through an increase in the fragility and destruction of red blood cells. This increased ferric state generates an oxidative stress environment (as evidenced by the Fenton and Haber-Weiss reactions), leading to the damage of nucleic acid as well as to the alteration of cellular proteins.[18] Low magnesium may also lead to a decrease in lysosomal integrity, thus promoting further oxidant generation.[3] Furthermore, low magnesium leads to concomitant decreases in intracellular zinc and copper levels, which disorganize microtubule and microfilament structure. Copper deficiency may decrease the activity of antioxidative enzymes such as cellular superoxide dismutase activity. Finally, magnesium deficiency may lead to a decrease in tissue vitamin E levels that may favor lysis of cells through oxidative damage.

Increased endothelial cytotoxicity as a result of intracellular reactive oxidation products may contribute to vascular injury, which, in turn, induces endothelial proliferation and vasospasm during magnesium deficiency. Dickens et al[19] demonstrated that isolated magnesium-deficient cells produced two- to threefold higher levels of thiobarbituric acid–reactive materials when exposed to the free radical generators dihyroxyfumarate and ferric iron-adenosine diphosphate (Fe^{3+}-ADP). Furthermore, the magnesium-deficient cells exhibited a time-dependent increase in fluorescence, suggestive of intracellular lipid peroxidation.

The loss of cellular viability as a result of magnesium deficiency–induced cytotoxicity that has been reported has led to numerous studies that seek to define the protective action of magnesium on free radical generation. One possibility for the protective action of magnesium on free radical generation is the inhibition of adenosine deaminase, an enzyme involving adenine nucleotide catabolism. The inhibition of this enzyme reduces the concentration of hypoxanthine, the substrate for xanthine oxidase, therefore reducing excessive superoxide anion production. Specifically, Mak et al[18] demonstrated that magnesium-gluconate, but not other magnesium salts, significantly inhibited the formation of hydroxyl free radicals. Also, magnesium gluconate dose dependently inhibited the iron catalyzed nucleic acid degradation; the magnesium gluconate salt displaces iron from the catalytic subunits of enzymes involved with oxidative damage.[4]

Magnesium Deficiency–Induced Inflammatory Response

Another cause of magnesium deficiency-induced free radical formation is the amplification of proinflammatory products such as circulating histamine, interleukin-1 (IL-1), IL-6, tumor necrosis factor a, and endothelin. Wiles et al[20] explained that both acute and chronic effects of magnesium deficiency may induce inflammatory change in vascular smooth muscle. In essence, studies suggest that magnesium deficiency may result in a deficient wound healing response and collagen synthesis involving oxidative injury and growth stimulation in the vascular system.[21]

Conclusion

Multiple vaso-occlusive mechanisms beyond hypomagnesemia may contribute to vasospasm. However, magnesium-mediated vascular protection provides a practical clinical mechanism to enhance recovery from and to provide protection against the deleterious sequelae of SAH-induced vasospasm.

REFERENCES

1. Treggiari MM, Romand JA, Martin JB, Reverdin A, Rufenacht DA, de Tribolet N. Cervical sympathetic block to reverse delayed ischemic neurological deficits after aneurysmal subarachnoid hemorrhage. Stroke 2003;34:961–967
2. Freedman JC. Biophysical chemistry of cellular electrolytes. In: Sperelakis N, ed. Cell Physiology: Source Book. San Diego: Academic; 1995;3–17
3. Chakraborti S, Chakraborti T, Mandal M, Mandal A, Das S, Ghosh S. Protective role of magnesium in cardiovascular diseases: a review. Mol Cell Biochem 2002;238:163–179
4. Barile M, Van De WF, Mbia JJ, et al. Intravenous magnesium sulfate administration in a patient with refractory vasospasm following subarachnoid hemorrhage. Intensive Care Med 2003;29:1182–1185
5. Boet R, Mee E. Magnesium sulfate in the management of patients with Fisher grade 3 subarachnoid hemorrhage: a pilot study. Neurosurgery 2000;47:602–606
6. Brewer RP, Parra A, Lynch J, Chilukuri V, Borel CO. Cerebral blood flow velocity response to magnesium sulfate in patients after subarachnoid hemorrhage. J Neurosurg Anesthesiol 2001;13:202–206
7. Chia RY, Hughes RS, Morgan MK. Magnesium: a useful adjunct in the prevention of cerebral vasospasm following aneurysmal subarachnoid haemorrhage. J Clin Neurosci 2002;9:279–281
8. Veyna RS, Seyfried D, Burke DG, et al. Magnesium sulfate therapy after aneurysmal subarachnoid hemorrhage. J Neurosurg 2002;96:510–514
9. Mori T, Nagata K, Ishida T, et al. Sequential morphological changes of the constrictive basilar artery in a canine model of experimental cerebral vasospasm by talc injection. J Vet Med Sci 1994;56:535–540
10. Pyne GJ, Cadoux-Hudson TA, Clark JF. Magnesium protection against in vitro cerebral vasospasm after subarachnoid haemorrhage. Br J Neurosurg 2001;15:409–415
11. Ram Z, Sadeh M, Shacked I, Sahar A, Hadani M. Magnesium sulfate reverses experimental delayed cerebral vasospasm after subarachnoid hemorrhage in rats. Stroke 1991;22:922–927
12. Kawanabe Y, Masaki T, Hashimoto N. Effects of the Ca^{2+}-permeable nonselective cation channel blocker LOE 908 on subarachnoid hemorrhage-induced vasospasm in the basilar artery in rabbits. J Neurosurg 2003;98:561–564
13. Bara M, Guiet-Bara A. Magnesium regulation of Ca^{2+} channels in smooth muscle and endothelial cells of human allantochorial placental vessels. Magnes Res 2001;14:11–18
14. Yang ZW, Wang J, Zheng T, Altura BT, Altura BM. Low $[Mg^{2+}]$ induces contraction and $[Ca^{2+}]$ rises in cerebral arteries: roles of Ca^{2+}, PKC and PI_3. Am J Physiol Heart Circ Physiol 2000; 279:H2898–H2907
15. Dunwiddie TV, Masino SA. The role and regulation of adenosine in the central nervous system. Annu Rev Neurosci 2001; 24:31–55
16. Borel CO, McKee A, Parra A, et al. Possible role for vascular cell proliferation in cerebral vasospasm after subarachnoid hemorrhage. Stroke 2003;34:427–433
17. Kent TA, Jazayeri A, Simard JM. Calcium channels and nifedipine inhibition of serotonin-induced $[^3H]$thymidine incorporation in cultured cerebral smooth muscle cells. J Cereb Blood Flow Metab 1992;12:139–146
18. Mak IT, Komarov AM, Kramer JH, Weglicki WB. Protective mechanisms of Mg-gluconate against oxidative endothelial cytotoxicity. Cell Mol Biol (Noisy-le-Grand) 2000;46:1337–1344
19. Dickens BF, Weglicki WB, Li YS, Mak IT. Magnesium deficiency in vitro enhances free radical-induced intracellular oxidation and cytotoxicity in endothelial cells. FEBS Lett 1992;311:187–191
20. Wiles ME, Wagner TL, Weglicki WB. Effect of acute magnesium deficiency (MgD) on aortic endothelial cell (EC) oxidant production. Life Sci 1997;60:221–236
21. Shivakumar K, Kumar BR. Magnesium deficiency enhances oxidative stress and collagen synthesis in vivo in the aorta of rats. Int J Biochem Cell Biol 1997;29:1273–1278

11

Pathogenesis of Cerebral Vasospasm: The Role of Cerebral Micro circulatory Changes

SHIGEHARU SUZUKI, M.D. AND HIROKI OHKUMA, M.D.

Cerebral ischemia during cerebral vasospasm associated with aneurysmal subarachnoid hemorrhage (SAH) has been attributable mainly to the persistent luminal narrowing of the major cerebral arteries. However, there are some cases in which the degree of major artery luminal narrowing does not always correspond to the ischemic symptoms. We have investigated factors other than major arterial luminal narrowing that may be affecting cerebral ischemia during cerebral vasospasm after SAH (Table 11–1).

Role of Platelet Hyperativity in Cerebral Ischemia during Cerebral Vasospasm

Cerebral Microthrombosis in Symptomatic Vasospasm

We suggested a role for cerebral microthrombosis, perhaps related to platelet hyperactivity, in symptomatic vasospasm based on the following data. A comparative investigation of the pathogenetic factors contributing to symptomatic cerebral vasospasm was made by quantitative histological and clinical studies in four patients who died while suffering from symptomatic cerebral vasospasm and in two who died without fatal cerebral vasospasm.[1,2] In four patients who died with symptomatic cerebral vasospasm, histological examination of the brain found many white and fibrin microthrombi together with changes consistent with ischemia and infarction in the territories of the spastic arteries in two cases. The changes corresponded to low-density areas observed on computed tomographic (CT) scans and to neurological symptoms typically associated with ischemia and infarction in these territories. In one case who had suffered severe vasospasm in both anterior cerebral and middle cerebral arteries, bilateral low-density areas were observed on CT scans, and multiple fibrin thrombi were seen diffusely throughout the brain. In this case, the possible complication of disseminated intravascular coagulation could not be ruled out. In the fourth case, extensive bilateral low-density areas (left greater than right) were observed on CT scans, and multiple microthrombi were seen diffusely but predominantly in the left cerebral hemisphere. Negligible thrombi were observed in two cases that died without fatal cerebral vasospasm and whose immediate cause of death was considered to be acute hydrocephalus and aneurysmal rerupture, respectively. The locations of microthrombi were

TABLE 11–1 Factors Other Than Luminal Narrowing of Major Arteries That May Contribute to Cerebral Ischemia after Aneurysmal SAH

Factor	Evidence	Prevention
Platelet hyperactivity	Cerebral microthrombosis in patients who die of symptomatic cerebral vasospasm	Antiplatelet drugs such as trapidil (pyrimidine derivative) and OKY-046 (imidazole derivative)
Changes in intraparenchymal small arteries	Luminal narrowing and wall thickness of small arteries after experimental SAH, prolonged cerebral circulation time in SAH patients	None known at present

significantly greater in the regions clinically identified to have been ischemic or infarcted.

Clinical Studies on Prevention of Cerebral Vasospasm with Thromboxane A_2 Synthesis Inhibitors

TRAPIDIL

The results of the preceding clinical studies, as well as additional experimental evidence, led us to hypothesize that subarachnoid focal acidosis resulting from anaerobic changes of subarachnoid clots may upset the balance of synthesis of thromboxane A_2 and prostaglandin I_2 by prostaglandin endoperoxides on the inner surface of cerebral arteries and be an important factor contributing to the pathogenesis of cerebral vasospasm. These changes would lead to a higher concentration of thromboxane A_2, a prostanoid that causes arterial contraction and platelet aggregation, and to vasospasm.

We tested the effects of trapidil, an antagonist and selective synthesis inhibitor of thromboxane A_2, in a series of 20 patients for the prevention of cerebral vasospasm and cerebral ischemia after aneurysmal rupture.[3] Vasospasm was demonstrated by angiography in nine of these cases, but only two of the nine showed mild signs of cerebral ischemia. Of the 20 patients, 15 were discharged from the hospital as cured and 3 had a neurological deficit at discharge. Our findings suggest the significance in symptomatic vasospasm of thrombus formation by platelet aggregation and the effectiveness of trapidil as a preventive measure.

COOPERATIVE STUDY OF OKY-046

The prevention of cerebral vasospasm with OKY-046, an imidazole derivative that inhibits thromboxane synthetase, was studied cooperatively in 10 neurosurgical services.[4] OKY-046 was administered as a continuous intravenous infusion of 2, 5, or 10 mU/kg/min beginning from the earliest possible day to the 14th day after SAH in 82 patients with ruptured cerebral aneurysms. Sixty-eight patients (83%) showed moderate- to high-density SAH on their initial CT scans. Angiographic vasospasm was seen in 58 patients, representing 71% of all cases or 81% of the 72 cases for which angiograms were available. The vasospasm was moderate to severe in 45 patients (55 or 63%). Symptomatic vasospasm occurred, however, in only 27 patients (33%) and in 18 of those cases, moreover, the symptoms were mild or transient. The condition of the patients at 1 month after the SAH was classified into nine grades from 0 (normal) to d (deceased). Fifty-two patients (63%) were classified as 0 or 1 and 64 (78%) as better than 3 (a grade compatible with unaided daily life). The administration of OKY-046 was demonstrated to decrease the level of thromboxane B_2 in the blood. This study emphasized the effectiveness of the drug for symptomatic vasospasm and supports our previous contention that cerebral microthrombosis may play an important role in the pathogenesis of cerebral vasospasm.

COOPERATIVE, DOUBLE-BLIND STUDY OF OKY-046

A double-blind study was conducted at 48 neurosurgical services in Japan to investigate the usefulness of OKY-046, an imidazole derivative and thromboxane synthetase inhibitor, on cerebral vasospasm and cerebral ischemic symptoms in patients with ruptured cerebral aneurysms.[5] OKY-046 was administered in two daily doses of 80 mg (low-dose group) or 400 mg (high-dose group), and compared with a group given a placebo (placebo group). The occurrence of cerebral vasospasm was significantly lower in the low-dose group than in the placebo group, and the development of low-density areas on CT scans was significantly lower in both the low- and high-dose groups than in the placebo group. Motor paralysis in the low-dose group improved significantly sooner, and that in the high-dose group tended to improve sooner than that in the placebo group. In subjects with severe vasospasm, the incidence of low-density areas on CT scans was significantly lower, with better functional prognosis, in the low-dose group than in the placebo group. In subjects with severe grades on the Glasgow coma scale, Japan coma scale, or high density score, the functional prognosis at 1 month after the aneurysmal rupture was significantly better in the low-dose group than in the placebo group, though no significant differences were seen in the overall outcome. There were no significant differences among the three groups in the development of a variety of laboratory-determined abnormalities or of adverse reactions. It is thus concluded that OKY-046 is clinically useful at a dose of 80 mg/day for cerebral vasospasm and cerebral ischemic symptoms after SAH caused by aneurysmal rupture.

Changes of Intraparenchymal Small Vessels after SAH

Experimental Study

Changes in the intraparenchymal small vessels after SAH were examined in the canine two-hemorrhage model.[6,7] Three, 7, or 14 days after the first SAH, the dogs were sacrificed by perfusion-fixation. Specimens for morphological examination were processed by one of two methods. In the first, after perfusion-fixation, polyester resin (Mercox CL2C, Dainippon Ink and Chemical, Tokyo, Japan) was injected through the angiography catheter. The brain was removed, and the

blocks of tissue were dissected from both the anterior Sylvian gyri and the pons. The block was kept in a 20% NaOH solution to corrode away brain tissue surrounding the plastic cast, and the cast was examined by scanning electron microscopy (Hitachi S-405, Hitachi, Tokyo, Japan). For the second method, after perfusion-fixation, the anterior Sylvian gyri and the pons were removed as a 10 mm cubic block and immersed in 2.5% glutaraldehyde in 0.1 mol/L phosphate buffer (pH 7.4). The block was dehydrated, embedded in paraffin, and sectioned at 4 μm from the brain surface to a depth of 1000 μm. The sections were stained by the van Gieson, elastica van Gieson, and hematoxylin and eosin staining techniques and examined by light microscopy at a magnification of 200 to 400 ×. The same processing techniques were used to examine arteries from a control group in which physiological saline was injected into the cisterna magna twice and who were sacrificed 7 days later, and a completely untreated normal group.

Corrosion casts that were examined by scanning electron microscopy revealed that the arterioles originating from parenchymal arteries reached 700 to 800 μm in depth from the brain surface and had a gradually reducing width in control animals and untreated normal animals. A well-developed capillary network was observed. Three to 7 days after SAH, the arterioles showed tapered narrowing, and capillary networks became scarce. Fourteen days after SAH, these findings improved, returning to a normal appearance. The perforating arteries originating from the basilar artery showed regular and smooth surface both at their proximal portion and at their peripheral portion in the control and normal dogs. Three to 7 days after SAH, the perforating arteries showed the same findings at their cisternal portion as that of control and normal dogs; however, irregular width with external folds was seen in their intraparenchymal portion. Fourteen days after SAH, these findings improved.

Sectioned slices examined by light microscopy revealed that the wall of the intraparenchymal portion of the arterioles originating from parenchymal arteries became thick and their lumens became narrow 3 to 7 days after SAH compared with the normal and control groups. Fourteen days after SAH, these findings improved. The perforating arteries originating from the basilar artery showed the wall became obviously thick, and the collagenous fibers in the adventitia and the perivascular space increased 7 days after SAH compared with the normal and control groups.

CLINICAL STUDY

Cerebral microcirculatory changes during cerebral vasospasm after aneurysmal SAH are still controversial and uncertain. This study investigated the changes of cerebral microcirculation during cerebral vasospasm and clarified the roles of microcirculatory disturbances in cerebral ischemia by measuring cerebral circulation time and regional cerebral blood flow.[8]

In 24 cases with aneurysmal SAH, regional cerebral blood flow studies were obtained using single-photon emission CT. Digital subtraction angiography was performed on the same day between 5 and 7 days after SAH and/or within 4 hours after the onset of delayed ischemic neurological deficits. Cerebral circulation time was obtained by analyzing the time–density curve of the contrast media on digital subtraction angiography images and was divided into proximal cerebral circulation time, which was defined as the circulation time through the extraparenchymal large arteries, and peripheral cerebral circulation time, which was defined as the circulation time through the intraparenchymal small vessels. They were analyzed in association with regional cerebral blood flow and angiographic vasospasm.

Severe angiographic vasospasm was associated with a statistically significant decrease in regional cerebral blood flow. There was a significant correlation between the degree of angiographic vasospasm and the regional cerebral blood flow ($r = 0.43$, $p = .0006$). Peripheral cerebral circulation time showed a strong inverse correlation with regional cerebral blood flow ($r = –0.77$, $p < .0001$). Even in patients with no or mild to moderate angiographic vasospasm, prolonged peripheral cerebral circulation time clearly was associated with decreased regional cerebral blood flow. The results suggest that in addition to the marked luminal narrowing of large arteries detected as severe angiographic vasospasm, microcirculatory changes detected as prolonged peripheral cerebral circulation time caused cerebral ischemia during cerebral vasospasm. These results suggested that impaired autoregulatory vasodilation or decreased luminal caliber in intraparenchymal vessels may take part in cerebral ischemia during cerebral vasospasm.

REFERENCES

1. Suzuki S, Suzuki M, Iwabuchi T, Kamata Y. Role of multiple cerebral microthrombosis in symptomatic cerebral vasospasm: with a case report. Neurosurgery 1983;13:199–203
2. Suzuki S, Kimura M, Souma M, Ohkuma H, Shimizu T, Iwabuchi T. Cerebral microthrombosis in symptomatic cerebral vasospasm: a quantitative histological study in autopsy cases. Neurol Med Chir (Tokyo) 1990;30:309–316
3. Suzuki S, Sobata E, Iwabuchi T. Prevention of cerebral ischemic symptoms in cerebral vasospasm with trapidil, an antagonist and selective synthesis inhibitor of thromboxane A2. Neurosurgery 1981;9:679–685
4. Suzuki S, Iwabuchi T, Tanaka T, et al. Prevention of cerebral vasospasm with OKY-046 an imidazole derivative and a thromboxane synthetase inhibitor: a preliminary cooperative clinical study. Acta Neurochir (Wien) 1985;77:133–141

5. Susuki S, Sano K, Handa H, et al. Clinical study of OKY-046, a thromboxane synthetase inhibitor, in prevention of cerebral vasospasms and delayed cerebral ischaemic symptoms after subarachnoid haemorrhage due to aneurysmal rupture: a randomized double-blind study. Neurol Res 1989;11:79–88
6. Ohkuma H, Itoh K, Shibata S, Suzuki S. Morphological changes of intraparenchymal arterioles after experimental subarachnoid hemorrhage in dogs. Neurosurgery 1997;41:230–236
7. Ohkuma H, Suzuki S. Histological dissociation between intra- and extraparenchymal portion of perforating small arteries after experimental subarachnoid hemorrhage in dogs. Acta Neuropathol (Berl) 1999;98:374–382
8. Ohkuma H, Manabe H, Tanaka M, Suzuki S. Impact of cerebral microcirculatory changes on cerebral blood flow during cerebral vasospasm after aneurysmal subarachnoid hemorrhage. Stroke 2000;31:1621–1627

12

Bilirubin Oxidation Products and Their Possible Role in Subarachnoid Hemorrhage–Induced Cerebral Vasospasm

JOSEPH F. CLARK, PH.D., GAIL J. PYNE-GEITHMAN, D.PHIL., MELISSA A. LYONS, PH.D., SUSAN J. BIEHLE, PH.D., FRANK R. SHARP, M.D., PH.D.

Abstract

Cerebral vasospasm occurs in about half of all subarachnoid hemorrhage (SAH) patients. Our recent research has shown that bilirubin oxidation products (BOXs) are present in the cerebrospinal fluid (CSF) of SAH patients with vasospasm, and that BOXs can contribute to vasoconstriction and vasospasm in vitro and in vivo. Cisternal injections of BOXs in vivo caused 6 of 10 rats to die within 10 minutes, whereas 12 of 12 rats survived for 24 hours following blood injections. We found that an extractable substance in the CSF of SAH patients inhibits vascular smooth muscle phosphatase in vitro. Although the events leading to the vasospasm are not understood, a component of the occlusion may be due to vascular remodeling. Because of the ability to model vasospasm with phosphatase inhibition, we investigated the actions of BOXs, okadaic acid (a phosphatase inhibitor), and phorbol-12 myristate-13 acetate (PMA), a protein kinase activator, on vascular smooth muscle cell morphology and metabolism. Immunohistochemistry was used to assess morphology and a-smooth muscle actin distribution in smooth muscle cells 4 days after the application of these substances. Lactate dehydrogenase release was measured as an indicator of cell stress and oxidative metabolism to assess metabolic activity. We found that okadaic acid, PMA, or BOXs caused vascular smooth muscle cells to lose their normal spindle shape, with a-actin fiber distribution being strikingly altered. These morphological changes are consistent with pathological remodeling of the cells. We found that okadaic acid, PMA, and BOXs increased lactate dehydrogenase release ($p < .001$), which is an index of cell stress. Oxidative metabolism significantly increased in response to low-dose (1:20) and high-dose (1:12) BOXs (143 ± 9%, and 180 ± 12%, respectively, $p < .001$). PMA and okadaic acid also caused a significant and dose-dependent increase in metabolic activity. We conclude that BOXs alter normal morphology, change a-actin distribution patterns, and increase metabolic activity in cultured vascular smooth muscle cells and that these observations are consistent with vascular remodeling playing a role in SAH-induced cerebral vasospasm. The ability of okadaic acid and PMA to emulate some of these changes may provide insight into the putative mechanisms of BOXs. Although the mechanism remains unclear, our results suggest that BOXs could play a role in the vascular remodeling that occurs following SAH.

We and others have reported that blood products and vasoconstrictors may play a role in SAH-induced cerebral vasospasm.[12] Data from studies in vitro demonstrate that CSF from SAH-induced cerebral vasospasm patients is vasoactive and alters the vascular smooth muscle contraction–relaxation cycle. These data also suggest that the breakdown of bilirubin is associated with cerebral vasospasm as well. Recently we found that bilirubin oxidation products (BOXs) potentiate vasoconstriction and that these molecules are found in the CSF of SAH patients.[1,3–5] There have been three BOXs identified; BOX A, BOX B, and 4-methyl-3-vinylmaleimide: We are working to determine the role of BOXs in the pathogenesis of SAH-induced cerebral vasospasm and demonstrate their actions on vascular smooth muscle.

Vascular Metabolic and Contractile Function and Dysfunction

Normal vascular function is essential for adequate perfusion to all vascular beds. When vascular function is impaired, hypotension, hypertension, ischemia, or hemorrhage can occur. These studies[6–16] have developed a large body of information with regard to the contractile, metabolic, bioenergetic, and oxidative function and dysfunction of vascular smooth muscle during diseases such as hypertension, heart failure, and stroke.

We have reported that energy failure can be associated with increased levels of adenosine diphosphate (ADP) in the vascular smooth muscle, and importantly found that alterations in ADP concentration can impair and inhibit relaxation and relaxation kinetics.[1,8,9,12,15] It was proposed that mitochondrial failure might be associated with vascular dysfunction.[11-17] We also reported that mitochondrial failure can cause or contribute to cardiovascular dysfunction.[18] The observation of metabolic and mitochondrial dysfunction with hypertension and heart failure has been expanded when we reported that vascular mitochondrial enzymes are altered during severe hypertension and hypertrophy.[13]

Analysis of CSF and Vascular Dysfunction in SAH-Induced Cerebral Vasospasm

An in vitro model for vasoconstriction was developed using porcine carotid artery rings to assess the metabolic and contractile effects of extracted human CSF of cerebral vasospasm patients.[15,17,19–22] Oxygen consumption measurements showed that CSF from control patients had no effect on oxygen consumption of the porcine carotid artery. However, the carotids exposed to the CSF from SAH patients with vasospasm (CSF_V) showed a marked increase in oxygen consumption and a decrease in the rate of relaxation.

Phosphatase Inhibition Increases Oxygen Metabolism and Promotes Constriction of the Carotid Artery

Okadaic acid is a protein phosphatase 2A inhibitor and its application to vascular smooth muscle results in inhibition of multiple enzymes including the enzymes controlling the contractile proteins. We compared the vessels' responses to CSF and okadaic acid and found that okadaic acid and CSF_V both produced significant and similar stimulation of smooth muscle oxygen consumption. The technique of measuring oxygen consumption in the vascular smooth muscle provides a metabolic index of the vessel's function and is an indirect index of adenosine triphosphate (ATP) utilization.[4,15,21,23,24]

In experiments where we monitored the oxygen consumption of porcine carotid artery rings exposed to okadaic acid or CSF_V, we see increased oxygen consumption and slower relaxation times. When the okadaic acid treatment experiments were compared with the results from CSF_V, the responses were quite similar. Application of CSF_V also increased oxygen consumption and decreased relaxation rates in vessels.[21] Dobutamine and norepinephrine partially protect against the increases of vascular smooth muscle oxygen metabolism produced by CSF_V. We have also reported a significant shift in the relationship between oxygen consumption and tension generation when tension is stimulated by CSF_V.[17,22] This is indicative of an increase in contractile energy costs because of an increase in the initial oxygen consumption rates at lower tensions. Whatever the functional changes that these data indicate, we believe that they demonstrate a pathological change in the blood vessels when exposed to CSF_V from patients with vasospasm.

Phosphatase Inhibitor in CSF of Patients with Vasospasm

As already presented, both CSF_V and okadaic acid stimulate oxygen consumption in porcine carotid arteries. This observation led us to postulate that the substance in CSF that stimulated oxygen consumption of the carotids might produce phosphatase inhibition similar to that observed with okadaic acid. A component of the effect of the CSF_V may indeed be a direct inhibition of the smooth muscle phosphatase, but we have yet to determine if the extracted CSF_V may also act via kinase activation, as proposed by Sato et al.[2,25]

Oxygen Consumption and Tension Caused by CSF_V Correlate with Clinical Grade

We examined the tension generation of porcine carotid arteries after application of CSF from SAH patients *(n* = 16).[17,22] CSF that stimulated oxygen consumption above a rate of 0.4 µmol/min/g dry weight was classified as CSF_V for these experiments (n = 8), and CSF that stimulated oxygen consumption below this level, or did not stimulate respiration, was classified as CSF_n (n = 8). To determine if these in vitro results correlated with the clinical condition of the patients, we demonstrated that the CSF_V patients had a Fisher grade of 3.2 ± 0.9 versus 2.3 ± 0.8 ($p < .05$) for CSF_n patients. Thus the oxygen consumption and tension measurements correlate with the clinical grade of the patients.[1,22]

Mitochondrial Uncoupling Is Not an Explanation

Though we have proposed that the increases in oxygen consumption are due to increases in myosin ATPase activity, it might also be possible that the compounds we have isolated uncouple oxidative phosphorylation in smooth muscle mitochondria. Therefore, we have isolated smooth muscle mitochondria and applied BOXs and CSF_V to the isolated mitochondria. At all doses tested we saw no significant change in oxygen consumption by the isolated mitochondria exposed to BOXs or CSF (data not shown). Therefore, mitochondrial uncoupling does not appear to explain the effects of CSF_V.

BOXs as a Possible Contributor to Cerebral Vasospasm

Similarities between BOXs and Extracted CSF from SAH Patients

We first determined whether peroxidation of bilirubin would produce chemical compounds similar to those that we had characterized physiologically in the CSF of SAH patients with vasospasm. Currently, we make BOXs by peroxidizing bilirubin and extracting the BOXs.[5] An interesting and important similarity between the vasoactivity of CSF_V and the peroxidized bilirubin is that both lose their activity when exposed to light (data not shown). Not only is this an important chemical similarity, it also may have represented a severe problem when previous investigators tried to isolate the vasoactive compound from these patients because the BOXs would have been lost during purification.[2627]

The next set of experiments performed demonstrated that BOXs produce prolonged vasospasm in vivo. To do this we developed a cranial window technique (Fig. 12–1). This cranial window technique

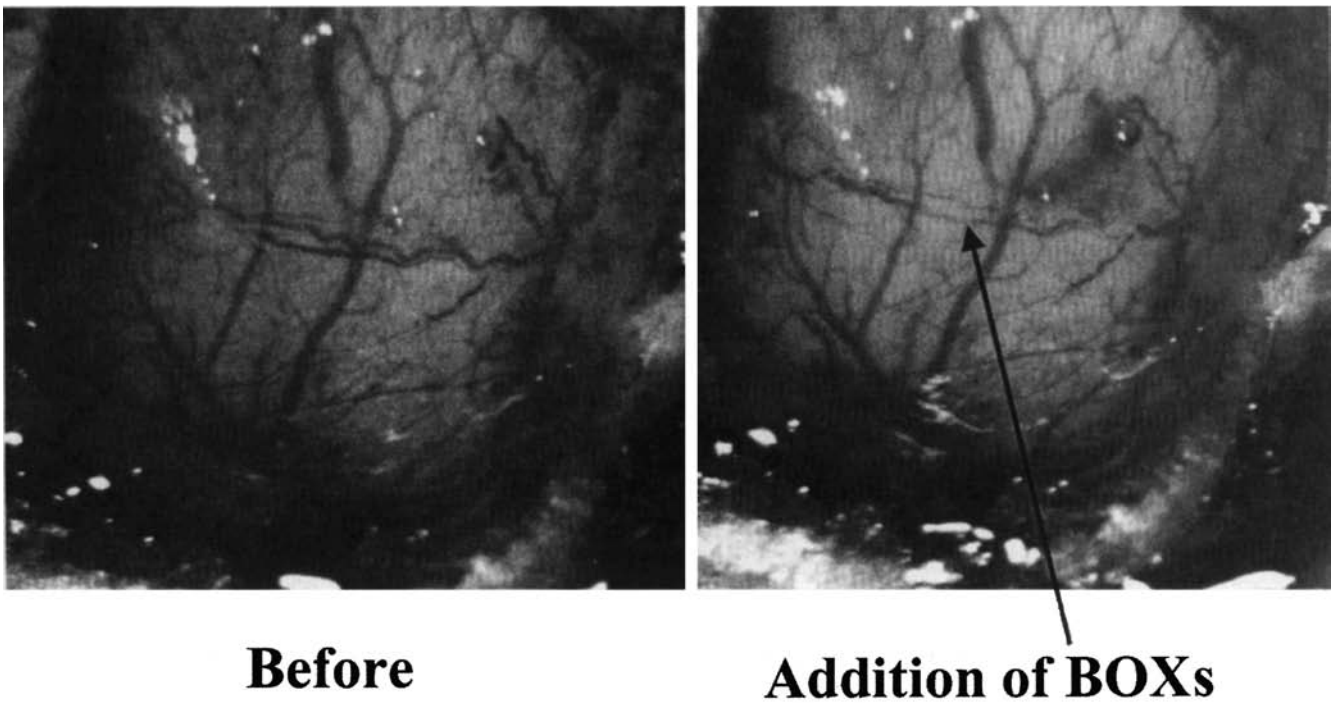


FIGURE 12–1 Photomicrographs of effects of bilirubin oxidation products (BOXs) on rat cortical vessels. Left panel shows control vessels on cortical surface of the rat brain before application of BOXs. Right panel shows that 40 minutes after topical application of BOXs there is prominent vasoconstriction of many arteries.

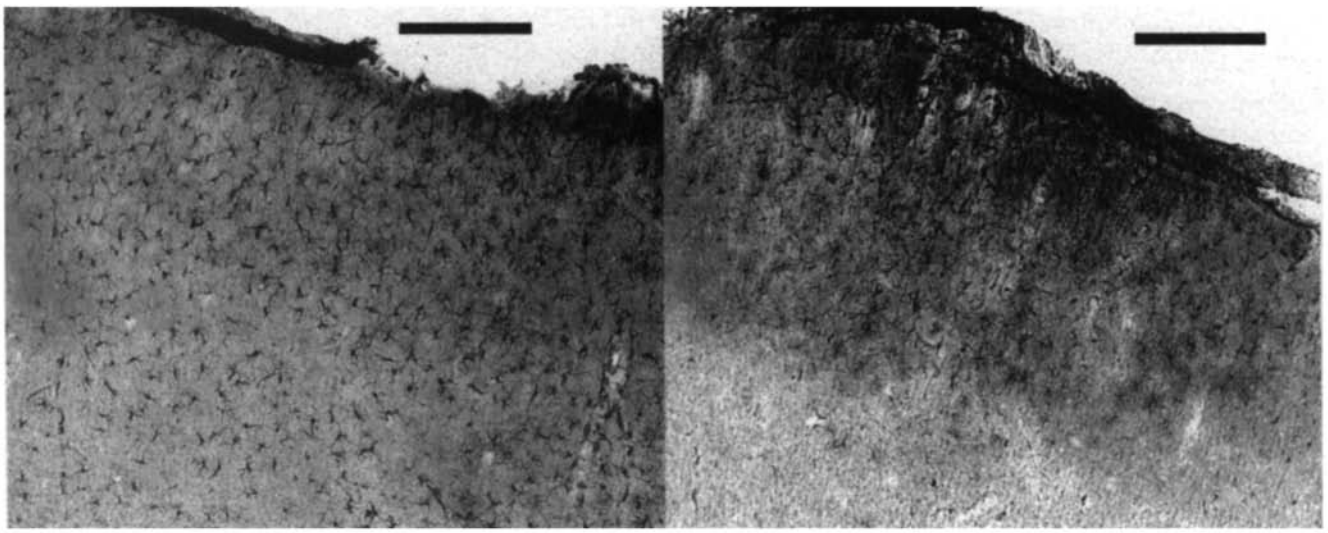


FIGURE 12–2 Application of pure bilirubin oxidation products (BOXs) A (left) and BOX B (right) caused a potent induction of heat shock protein 32 (also known as heme oxygenase-1) expression (dark staining) in the cortex of the rat brain. Photomicrographs are from two different representative rat brains where BOXs were applied to the surface of the cortex. Both produced visible vasospasm (not shown) and cortical heme oxygenase-1 induction 24 hours after application. Calibration bar is 200 μm.

allows us to follow the time course of vasospasm of vessels visible on the surface of the brain. Blood products have been implicated in the pathogenesis of SAH-induced cerebral vasospasm.[28–31] Extracts of CSF from SAH patients with vasospasm have been reported to be vasoactive and molecules contained within the CSF_V have been proposed to be the cause of cerebral vasospasm.[1,4,5,10] Interestingly, the time course for cerebral vasospasm following SAH closely correlates with the appearance of bilirubin in the CSF of SAH patients, although bilirubin alone does not cause vasospasm.[32,33] The BOXs (both BOX A and B) also appeared to produce stress gene expression in the brain following their application to the cortex through the cranial window (Fig. 12–2). Therefore, we proposed that a breakdown product of bilirubin might contribute to SAH-induced cerebral vasospasm.

Structures of These Molecules

We have performed a detailed structural analysis of the BOXs.[5] This was accomplished by focusing on isolating the molecules in the peroxidized bilirubin solution that stimulated vasoconstriction and increased oxygen consumption of carotid artery rings in vitro. Figure 12–3 shows the structures of the peroxidized bilirubin molecules that we have identified. BOX A and B have all of the characteristics shown previously for CSF from SAH patients with vasospasm (see all data regarding CSF_V). The BOXs stimulate oxygen consumption of vascular smooth muscle and cause vasoconstriction. The peroxidized bilirubin also altered vascular smooth muscle cell proliferation (data not shown). Therefore, these molecules could play an important role in the pathogenesis of vasospasm. The molecules characterized in Figure 12–3 were determined and confirmed using doping of high-pressure liquid chromatography–mass spectroscopy, nuclear magnetic resonance, and infrared spectroscopy.[5] These results strongly suggest that the peroxidized bilirubin compounds produced by the incubation of hydrogen peroxide with bilirubin in vitro are also present in CSF_V.

Delayed Vasospasm

The production of BOXs following SAH can explain the fact that SAH-induced cerebral vasospasm is often delayed many days to a week following the hemorrhage.[29,34,35] This is because it takes time for heme to be metabolized to bilirubin, with CSF bilirubin levels typically peaking at 3 to 5 days following SAH.[29,31,34] Others have noted that bilirubin concentrations in the CSF are closely related to the time course of vasospasm.[32,36] Using a pig model of SAH, we found that the production of bilirubin in the CSF correlates to a delayed time course of SAH-induced cerebral vasospasm (data not shown). Therefore, we believe that

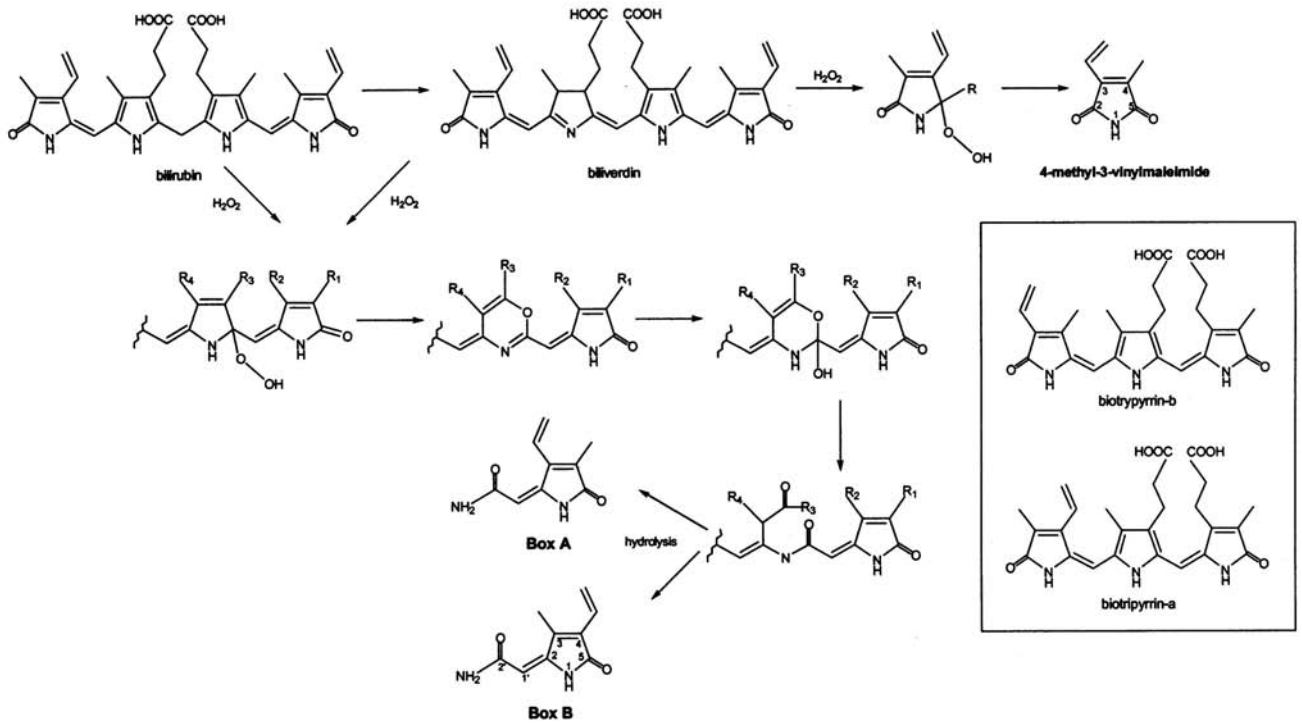


FIGURE 12–3 Outline of possible pathways for H_2O_2-mediated formation of bilirubin oxidation products (BOXs) A, B, and 4-methyl-3-vinylmaleimide from bilirubin or biliverdin. In pathway leading to BOX A production: R_1-CH_3, R_2-$CH{=}CH_2$ R_3-CH_3, R_4-CH_2-CH_2-COOH. In pathway leading to BOX B formation: $R_1CH{=}CH_2$, R_2-CH_3, R_3-CH_3, R_4-CH_2-CH_2-COOH. In pathway leading to 4-methyl-3-vinylmaleimide: R-part of bilirubin molecule.

the delayed production of bilirubin and hence BOXs is quite consistent with the clinical observations of vasospasm.

BOXs in Cell Culture

We have previously reported that BOXs are present in the CSF of SAH patients with cerebral vasospasm and that they can contribute to acute vasoconstriction and vasospasm both in vitro and in vivo. To examine the more chronic effects of BOXs, we investigated the actions of BOXs, okadaic acid (a phosphatase inhibitor), and phorbol-12 myristate-13 acetate (PMA), a protein kinase activator, on primary vascular smooth muscle cell morphology and metabolism. Therefore, immunocytochemistry was used to assess smooth muscle cell morphology and contractile protein distribution 96 hours after the application of BOXs, okadaic acid, or PMA. This time point was selected based on its relevance to the time course of the clinical presentation of cerebral vasospasm that tends to occur 3 to 10 days after SAH. We also measured changes in the levels of lactate dehydrogenase release and oxidative metabolism. Our results demonstrated that BOXs, okadaic acid, or PMA disrupt normal vascular smooth muscle cell morphology and alter a-actin (a contractile protein) distribution. These treatments significantly increased lactate dehydrogenase release, which is an index of increased cell stress. Vascular smooth muscle cell oxidative metabolism was also significantly increased following the chronic application of BOXs, okadaic acid, or PMA. Thus, alterations in protein phosphatase/kinase cascades (via okadaic acid and PMA) or the presence of BOXs alters vascular smooth muscle cell morphology and metabolic activity.

Conclusion

We have found that bilirubin oxidation products are vasoactive in vivo and in vitro. These data are consistent with BOXs playing a role in the pathogenesis of SAH-induced cerebral vasospasm.

Acknowledgment

This work was supported by the National Institutes of Health (NS042308, NS42697, HL67186) and the American Heart Association.

REFERENCES

1. Pyne GJ, Cadoux-Hudson TA, Clark JF. The presence of an extractable substance in the CSF of humans with cerebral vasospasm after subarachnoid haemorrhage that correlates with phosphatase inhibition. Biochim Biophys Acta 2000;1474:283–290

2. Sato M, Tani E, Fujikawa H, Kaibuchi K. Involvement of rho-kinase-mediated phosphorylation of myosin light chain in enhancement of cerebral vasospasm. Circ Res 2000;87:195–200

3. Clark JF, Reilly M, Sharp FR. Oxidation of bilirubin produces compounds that cause prolonged vasospasm of rat cerebral vessels: a contributor to subarachnoid hemorrhage-induced vasospasm. J Cereb Blood Flow Metab 2002;22:472–478

4. Pyne GJ, Cadoux-Hudson TA, Clark JF. Cerebrospinal fluid from subarachnoid haemorrhage patients causes excessive oxidative metabolism compared to vascular smooth muscle force generation. Acta Neurochir (Wien) 2001;143:59–62

5. Kranc KR, Pyne GJ, Tao L, Claridge TDW. Oxidative degradation of bilirubin produces vasoactive compounds. Eur J Biochem 2000;267:7094–7101

6. Clark JF, Kemp GJ, Radda GK. The creatine kinase equilibrium, free [ADP] and myosin ATPase in vascular smooth muscle cross-bridges. J Theor Biol 1995;173:207–211

7. Boehm EA, Clark JF, Radda GK. Metabolite utilization and compartmentation in porcine carotid artery: a study using beta-guanidinopropionic acid. Am J Physiol 1995;268:C628–C635

8. Clark JF, Dillon PF. Phosphocreatine and creatine kinase in energetic metabolism of the porcine carotid artery. J Vasc Res 1995;32:24–30

9. Clark JF, Khuchua Z, Boehm E, Ventura-Clapier R. Creatine kinase activity associated with the contractile proteins of the guinea-pig carotid artery. J Muscle Res Cell Motil 1994;15:432–439

10. Cadoux-Hudson T, Pyne G, Clark J. Subarachnoid haemorrhage induced cerebral vasospasm: a subcellular perspective on the control of tension. Emerg Ther Targets 1999;3:439–452

11. Clark J, Kranc K. The role of the mitochondrion in smooth muscle cell fate choices of proliferation versus apoptosis during vascular and cardiovascular disease. Emerg Ther Targets 1999;3:513–525

12. Clark JF. The creatine kinase system in smooth muscle. Mol Cell Biochem 1994;133–134:221–232

13. Clark JF, Radda GK, Boehm EA. The effects of anti-hypertensive therapy on the structural, mechanical and metabolic properties of the rat aorta. J Muscle Res Cell Motil 2000;21:255–267

14. Clark JF, Dillon PF. Mechanical and metabolic toxicity of 3-(trimethylsilyl)propanesulfonic acid to porcine carotid arteries. Biochim Biophys Acta 1989;1014:235–238

15. Pyne GJ, Cadoux-Hudson TA, Clark JF. Magnesium protection against in vitro cerebral vasospasm after subarachnoid haemorrhage. Br J Neurosurg 2001;15:409–415

16. Brewster LM, Clark JF, van Montfrans GA. Is greater tissue activity of creatine kinase the genetic factor increasing hypertension risk in black people of sub-Saharan African descent? J Hypertens 2000;18:1537–1544

17. Pyne GJ, Cadoux-Hudson TA, Clark JF. Cerebrospinal fluid from subarachnoid haemorrhage patients causes excessive oxidative metabolism compared to vascular smooth muscle force generation. Acta Neurochir (Wien) 2001;143:59–63

18. Clark JF, Khuchua Z, Kuznetsoz AV, et al. Actions of the creatine analogue beta-guanidinopropionic acid on rat heart mitochondria. Biochem J 1994;300:211–216

19. Pyne GJ, Cadoux-Hudson TA, Clark JF. Platelets play an essential role in the aetiology of cerebral vasospasm after subarachnoid haemorrhage. Med Hypotheses 2003;60:525–530

20. Pyne GJ, Cadoux-Hudson TAD, Clark JF. The presence of an extractable substance in the CSF of humans with cerebral vasospasm after subarachnoid haemorrhage that correlates with phosphatase inhibition. Biochim Biophys Acta 2000;1474:283–290

21. Clark JF, Pyne GJ, Choutka J, et al. In vitro therapy with dobutamine, isoprenaline and sodium nitroprusside protects vascular smooth muscle metabolism from subarachnoid haemorrhage induced cerebral vasospasm. Acta Neurochir (Wien) 2001; 143:721–728

22. Cadoux-Hudson TA, Pyne GJ, Domingo Z, Clark JF. The stimulation of vascular smooth muscle oxidative metabolism by CSF from subarachnoid haemorrhage patients increases with Fisher and WFNS grades. Acta Neurochir (Wien) 2001;143:65–72

23. Thomson NF, Thornton S, Clark JF. The effects of placental extracts from normotensive and preeclamptic women on vasoconstriction and oxidative metabolism. Am J Obstet Gynecol 2000;183:206–210

24. Bearchell MC, Redman CW, Pyne GJ, Cadoux-Hudson T, Clark JF. Vascular smooth muscle oxygen consumption is reversibly stimulated by sera from women with preeclampsia. Am J Obstet Gynecol 1998;179:1534–1538

25. Shimokawa H, Sato M, Katsumata N, et al. Rho-kinase-mediated pathway induces enhanced myosin light chain phosphorylations in a swine model of coronary artery spasm. Cardiovasc Res 1999;43:1029–1039

26. Hughes JT, Schianchi PM. Cerebral artery spasm: a histological study at necropsy of the blood vessels in cases of subarachnoid hemorrhage. J Neurosurg 1978;48:515–525

27. Boullin D, Mohan J, Grahame-Smith D.G. Evidence for the presence of vasoactive substance (possible involved in the aetiology of cerebral arterial spasm) in cerebrospinal fluid from patients with subarachnoid haemorrhage. J Neurol Neurosurg Psychiatry 1976;39:756–766

28. Findlay JM, Macdonald RL, Weir BK. Current concepts of pathophysiology and management of cerebral vasospasm following aneurysmal subarachnoid hemorrhage. Cerebrovasc Brain Metab Rev 1991;3:336–361

29. Weir B, Macdonald RL, Stoodley M. Etiology of cerebral vasospasm. Acta Neurochir Suppl (Wien) 1999;72:27–46

30. Zhang H, Weir BK, Macdonald RL, et al. Mechanisms of $[Ca^{++}]_i$ elevation induced by erythrocyte components in endothelial cells. J Pharmacol Exp Ther 1996;277:1501–1509

31. Mayberg MR. Cerebral vasospasm. Neurosurg Clin N Am 1998;9:615–627

32. Duff TA, Feilbach JA, Yusuf Q, Scott G. Bilirubin and the induction of intracranial arterial spasm. J Neurosurg 1988;69:593–598

33. Trost GR, Nagatani K, Goknur AB, Haworth RA, Odell GB, Duff TA. Bilirubin levels in subarachnoid clot and effects on canine arterial smooth muscle cells. Stroke 1993;24:1241–1245

34. Macdonald RL Weir BK. A review of hemoglobin and the pathogenesis of cerebral vasospasm. Stroke 1991;22:971–982

35. Weir B. The pathophysiology of cerebral vasospasm. Br J Neurosurg 1995;9:375–390

36. Morooka H. Cerebral arterial spasm, II: Etiology and treatment of experimental cerebral vasospasm. Acta Med Okayama 1978; 32:39–49

13

Activation of Microglia After Experimental Subarachnoid Hemorrhage in Rats

RYUHEI KITAI, M.D., YUJI HANDA, M.D., PH.D.,
MASAKI ISHIDA, M.D., TAKAO NAKAGAWA, M.D.,
AKIRA TSUCHIDA, M.D., TOSHIHIKO KUBOTA, M.D., PH.D.

Abstract

Although it is reported that subarachnoid hemorrhage (SAH) is associated with microglial activation in brain tissue, the role of activated microglia in the pathogenesis of possible neuronal injury following SAH is still obscure. This study sought to determine whether microglial activation associated with SAH could contribute to brain injury after SAH. To accomplish this, we observed morphological changes in brain microglia following experimental SAH in rats and studied hemoglobin-induced cytokine release from microglial cells in culture. SAH was induced by a cisternal injection of 0.2 mL of arterial blood or an identical volume of hemoglobin solution (100 mg/mL) prepared from the lysed arterial blood of rats. The animals were fixed by perfusion, and their brains were removed and assessed by immunohistochemical staining at times up to 7 days after SAH. Hemoglobin (1 mg/mL) was added to the medium of microglial primary cell cultures that were obtained from neonatal rats. The concentrations of cytokines interleukin-1ß (IL-1ß) and IL-6 in the culture supernatant fluid and in the intracellular contents were determined by an enzyme-linked immunosorbent assay (ELISA) 6 and 24 hours after addition of hemoglobin. A large increase in OX-42 (activated microglial marker) immunoreactivity was seen throughout the brain 2 to 7 days after SAH, suggesting activation of microglia had occurred at these times. Immunohistochemical staining for NG2 (oligodendrocyte progenitor) showed increased expression of NG2 in microglia as well as oligodendrocytes and some types of neurons. ELISA revealed that hemoglobin significantly stimulated microglia to produce IL-1ß (mean: 510 pg at 6 hours and 790 pg at 24 hours) compared with the control culture (mean: 320 pg). These results indicated that hemoglobin from lytic subarachnoid blood clot could activate microglia to release cytokines leading to secondary brain injury following SAH. We hypothesize that this pathophysiological mechanism could be important in the pathogenesis of cognitive impairment in patients following SAH.

Recent advances in management of aneurysmal subarachnoid hemorrhage (SAH) have achieved lower mortality and morbidity However, patients who survive SAH often have subtle neurobehavioral problems even when they have no grossly apparent focal neurological deficits.[1] Although the pathogenesis of these problems has not yet been resolved, one of the possible mechanisms seems to be secondary neuronal damage elicited by subarachnoid clot via inflammatory reactions.[2-3] Microglia are known to be the first responding cells to a variety of central nervous system injuries.[4-5] Microglial cells synthesize and secrete several cytokines, which coordinate the inflammatory responses in the brain tissue.[4-5] These changes in the inflammatory environment may possibly determine the survival of neurons after SAH. The present study was designed to investigate the response of microglia to subarachnoid blood in experimental models in vitro and in vivo.

Materials and Methods

In Vivo Study

Experimental SAH was induced by injection of hemoglobin solution (0.2 mL, 100 mg/mL in phosphate-buffered saline) into the cisterna magna of adult Sprague-Dawley rats. The animals were sacrificed on day 3 or day 5 following SAH. The animals were perfused transcardially with fixative solution. Specimens of the brain were prepared for immunohistochemical staining for OX-42 and NG-2. OX-42 is a complement receptor 3 (CR3) that is expressed on activated microglia, and NG-2 is a chondroitin sulfate proteoglycan that is found on the surface of the class of glial cells within the developing and mature central nervous system that have the properties of oligodendrocyte progenitors.

In Vitro Study

Microglial cells were cultured from minced brain tissue obtained from neonatal Sprague-Dawley rats. After 2 weeks of culture, microglial cells were detached from the astrocyte monolayer by vigorous manual shaking. Floating microglial cells were collected and plated at 5×10^5 cells/mL in 96 well plates. The hemoglobin solution (1 µg/mL concentration) was added to the culture media. We used lipopolysaccharide (1 ng/mL) as a positive control to induce cytokine synthesis. The supernatant fluids were collected at 6, 24, 48, and 72 hours after addition of hemoglobin to the culture medium. Concentrations of interleukin (IL)-1ß, IL-6, and tumor necrosis factor-a (TNFa) was determined by enzyme-linked immunosorbent assay (ELISA).

Results

In Vivo Study

We focused our histological examination on sections through the hippocampus. In this region, abundant OX-42-positive activated microglia were observed in rats subjected to SAH, whereas few OX-42-positive cells with tiny cell processes were observed in the normal control brain tissue (Fig. 13–1). The activated microglial cell is characterized by increased OX-42 immunostaining and by stout and elongated processes. This pathological change was observed in the entire brain as well as the hippocampus. The number of NG2-positive cells also increased throughout the whole brain 3 and 5 days after SAH compared with normal control brain (see Fig. 13–1).

In Vitro Study

The morphology of microglia was examined under phase contrast microscopy. The majority of untreated microglial cells displayed round shape with tiny processes (resting form), whereas in the presence of hemoglobin, enlarged microglial cells extended multiple processes as an activated amoeboid phenotype. This appearance was reminiscent of that induced by stimulation with lipopolysaccharide (Fig. 13–2). ELISA demonstrated increased concentration of IL-1ß in the supernatant fluid of microglial cultures stimulated by hemoglobin. For example, the concentration of IL-1ß 24 hours after addition of hemoglobin was 220 ± 69 pg/mL (mean ± SD, $n = 3$) versus 120 ± 91 for control cultures. This increased further to 380 ± 205 at 48 hours after addition of hemoglobin versus 228 ± 60 in control cultures (Fig. 13–3). TNFa concentration in the culture supernatant fluid also showed significant increases in response to addition of hemoglobin. The time course of cytokine concentrations showed that IL-lß and TNFa started to increase within 6 hours and continued to increase up to 48 hours following addition of hemoglobin. No appreciable concentrations of IL-6 were detected in supernatant fluids from untreated microglia or after stimulation with hemoglobin. IL-1ß production at baseline and in response to hemoglobin was detected only in microglial cultures and not in cultured astrocytes (see Fig. 13–3).

Discussion

Some patients who survive aneurysmal SAH demonstrate a diffuse SAH-induced encephalopathy manifest by varying degrees of intellectual impairment.[1] Previous studies in our laboratory reported changes in neuronal metabolites in the brain following SAH at a chronic stage[2] and that there was an inflammatory

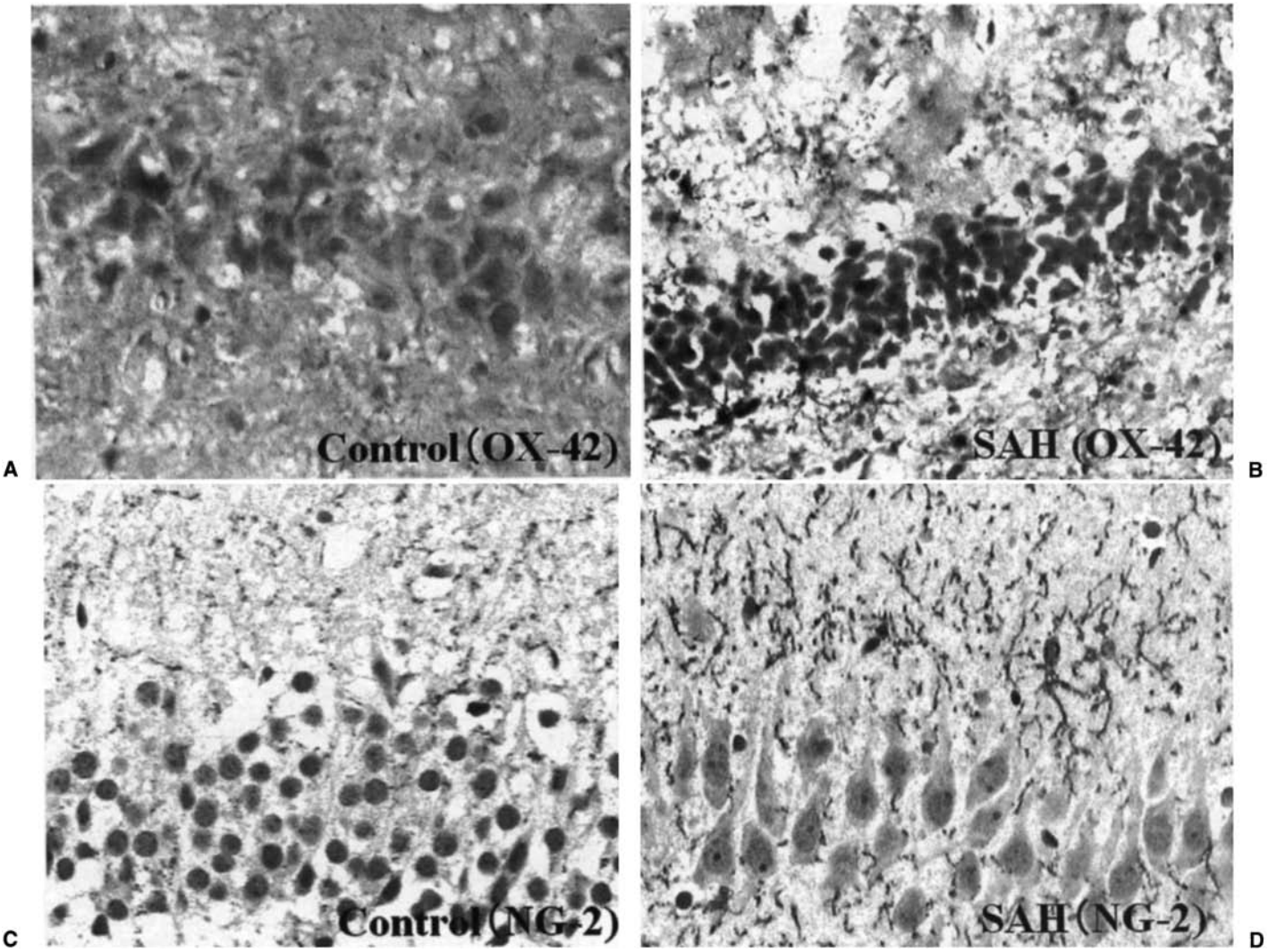


FIGURE 13–1 Photomicrographs of immunohistochemical staining of the rat hippocampus. (A) Normal control rats have only sparce staining of OX-42-positive cells with fine processes, whereas (B) 5 days after subarachnoid hemorrhage (SAH) there is robust staining of activated microglia with OX-42 in the hippocampus. (C) Similarly, control rats exhibit minimal immunoreactivity to NG-2, whereas (D) the hippocampus of rats 5 days after SAH shows numerous cells with immunoreactivity to NG-2.

response in the subarachnoid space and the brain after experimental SAH.[3] These clinical and experimental results suggest that neuronal injury occurs as a secondary injury following SAH. We hypothesize that it might be secondary to the inflammatory responses that appear to be elicted by subarachnoid blood clot. Microglia are the principal immune/inflammatory cells in the brain and have been recognized to be the first responders to injury in the central nervous system.[4,5] Hence, to clarify the pathogenesis of SAH-induced encephalopathy, it seems important to know whether and how microglia are activated by subarachnoid blood or its spasmogenic components, such as hemoglobin.

The present study demonstrated that brain microglia develop features of activated microglia after intracisternal injection of hemoglobin and that hemoglobin stimulated cultured microglia to release cytokines. Experimental studies have revealed that subarachnoid hemglobin may be taken up into microglia.[6,7] Although it is still obscure how heme proteins including hemoglobin and its breakdown products distribute into the brain, heme is potentially highly toxic because of its ability to intercalate into lipid membranes and to produce hydroxyl radicals.[8] Heme oxygenase-1 was induced in microglia throughout the brain after experimental SAH.[6,9] These results indicate that hemoglobin from subarachnoid blood

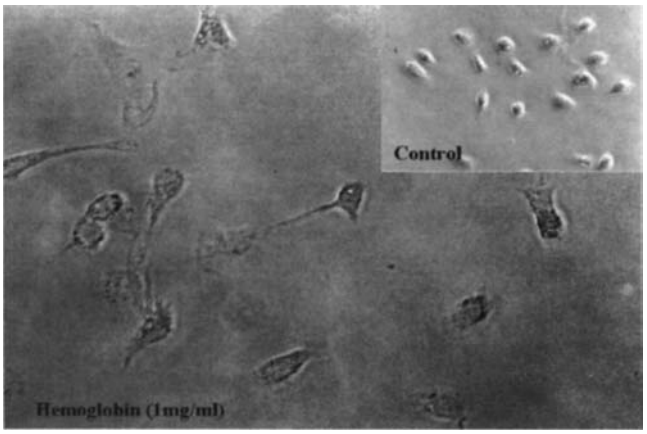


FIGURE 13–2 Photomicrographs of morphological changes in cultured microglia under phase contrast microscopy. Inset (upper right) shows untreated small round microglia with few processes. Main micrograph shows microglia after exposure to hemoglobin. These activated microglia have enlarged cytoplasm with elongated processes.

may penetrate into the brain parenchyma to interact or stimulate microglia to cause the first step in the inflammatory response.

IL-lß and TNFa are inflammatory cytokines. Increased levels of these cytokines have been implicated as key factors in the initiation of tissue damage in several different disorders.[10] Cytokine release might be a precursor to glial reactions such as activation of astrocytes. For example, IL-1 induces astrocytes to produce inducible nitric oxide synthase, resulting in the generation of high levels of nitric oxide. This has been suggested to contribute to the establishment of chronic inflammatory states through induction of additional cytokines.[10] In rodents, IL-1 is a mitogen for astrocytes in vitro, and intracerebral injection of IL-1 induces a reactive gliosis.[11] Furthermore, TNFa promotes proliferation of oligodendrocyte progenitors and remyelination.[12] Thus, microglia at different stages of activation may have different roles (protective or damaging) to other populations of brain cells including neurons, astrocytes, and oligodendrocytes. These inflammatory responses elicited subsequent to microglial activation following SAH might strongly influence neuronal survival or function and contribute to SAH-induced neuropathy that presents with varying degrees of cognitive impairment.

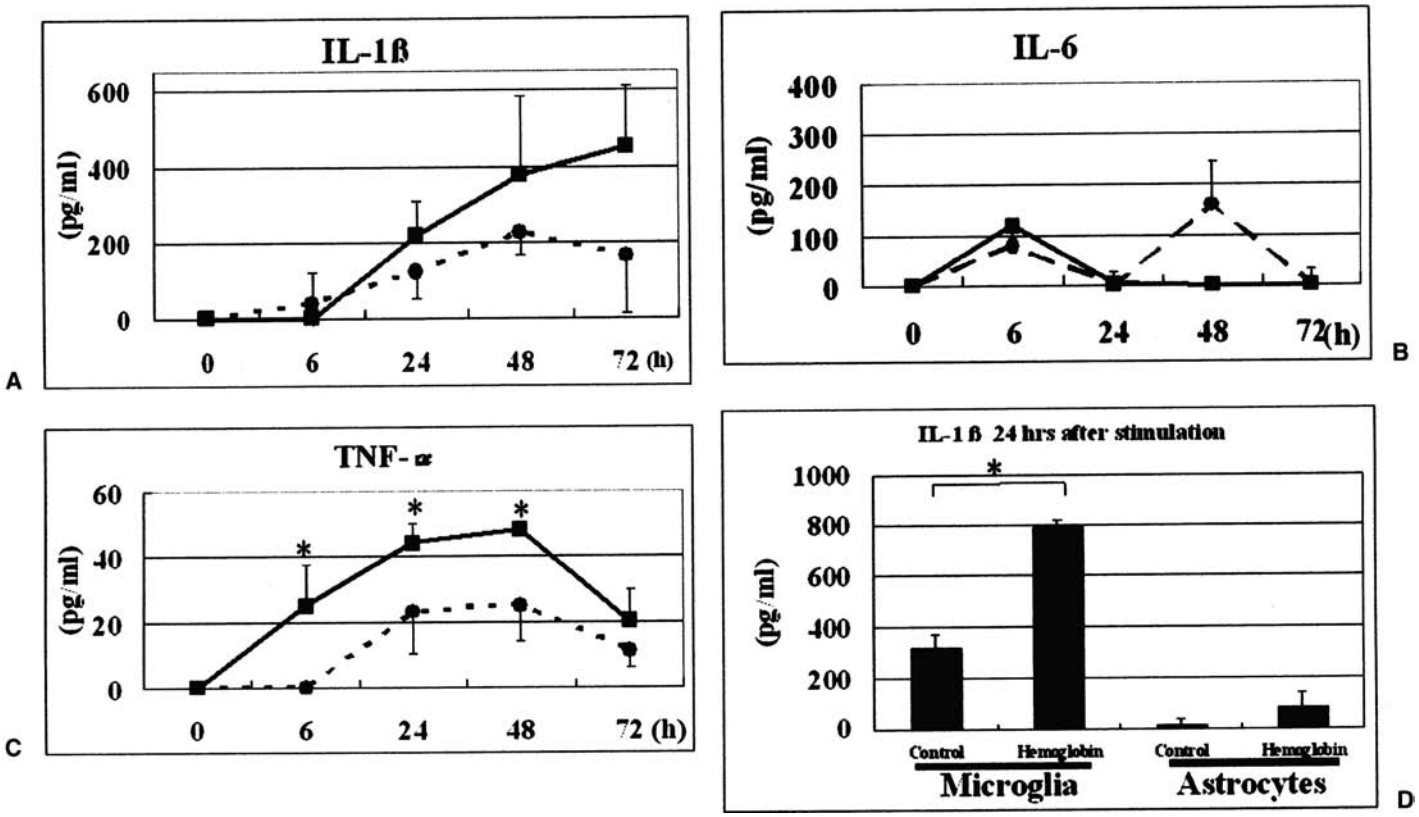


FIGURE 13–3 Graphs showing cytokine secretion from cultured microglia after addition of hemoglobin. (A) Interleukin (IL)-1ß and (C) tumor necrosis factor (TNF)a show significant increases in response to stimulation with hemoglobin, whereas (B) there were no appreciable changes observed in the concentration of IL-6. (D) IL-lß was detected exclusively in microglial cultures and was not detected in astrocyte cultures. Values are means ± standard deviation from triplicate wells (* $p < .05$).

REFERENCES

1. Ljunggren B, Sonesson B, Saveland H, Brandt L. Cognitive impairment and adjustment in patients without neurological deficits after aneurysmal SAH and early operation. J Neurosurg 1985;62:673–679
2. Handa Y, Kimura H, Arishima H, et al. Changes in neuronal metabolites in brain following subarachnoid haemorrhage evaluated by proton MR spectroscopy. Acta Neurochir Suppl 2001;77:135–138
3. Handa Y, Kubota T, Kaneko M, et al. Expression of intercellular adhesion molecule 1 (ICAM-1) on the cerebral artery following subarachnoid haemorrhage in rats. Acta Neurochir (Wien) 1995;132:92–97
4. Kato H, Walz W. The initiation of the microglial response. Brain Pathol 2000;10:137–143
5. Song X, Shapiro S, Goldman DL, et al. Fc Receptor I- and III-mediated macrophage inflammatory protein 1 induction in primary human and murine microglia. Infect Immun 2002; 70:5177–5184
6. Ozawa H, Nishida A, Mito T, et al. Immunohistochemical study of ferritin-positive cells in the cerebellar cortex with subarachnoidal hemorrhage in neonates. Brain Res 1994;651:345–348
7. Turner CP, Bergeron M, Matz P, et al. Heme oxygenase-1 is induced in glia throughout brain by subarachnoid hemoglobin. J Cereb Blood Flow Metab 1998;18:257–273
8. Tolosano E, Altruda F. Hemopexin: structure, function, and regulation. DNA Cell Biol 2002;21:297–306
9. Matz P, Turner C, Weinstein PR, et al. Heme-oxygenase-1 induction in glia throughout rat brain following experimental subarachnoid hemorrhage. Brain Res 1996;713:211–222
10. Liu JS, Amaral TD, Brosnan CF, et al. IFNs are critical regulators of IL-1 receptor antagonist and IL-1 expression in human microglia. J Immunol 1998;161:1989–1996
11. Giulian D, Woodward J, Young D, et al. Interleukin-1 injected into mammalian brain stimulates astrogliosis and neovascularization. J Neurosci 1988;8:2485–2490
12. Arnett HA, Mason J, Marino M, et al. TNF alpha promotes proliferation of oligodendrocyte progenitors and remyelination. Nat Neurosci 2001;4:1116–1122

14

Smooth Muscle Phenotype Change in Canine Basilar Artery After Experimental Subarachnoid Hemorrhage

MITSUO YAMAGUCHI, M.D.

Abstract

It has been unclear whether the mechanisms that sustain cerebral vasospasm after subarachnoid hemorrhage (SAH) in the acute stage are the same as those involved in the chronic stage. We hypothesized that smooth muscle phenotype change may play a role in prolonged vasospasm, and investigated functional and morphological changes in both stages. Vasospasm of the basilar artery was created using the two-hemorrhage canine model. Twenty-four dogs were divided into four groups to be euthanized 1,14,21, or 28 days after baseline angiography and SAH. Angiography was performed prior to SAH and on the day of sacrifice, and the chronological change of arterial diameter was examined. The basilar arteries from each of the four groups were studied to determine the maximal tension in response to high K+, relaxation to papaverine, and stiffness of the arterial wall. Morphological changes in microscopic transverse section and the collagen concentration of the basilar arteries were determined by a dye-binding method. Severe angiographic vasospasm was present on day 14 and had completely resolved by day 28. However, increased stiffness of the arterial wall, reduced contractility, and reduced relaxations were observed 14, 21, and 28 days after SAH. Abnormal round and shortened smooth muscle cells also were observed at all three times after SAH. The thickness of the adventitia and migration of fibrous tissue into the artery was remarkable on days 14 and 21. The collagen concentration increased on day 14 but was not significantly increased on days 21 and 28. In summary, there is a significant discrepancy between chronological changes of angiographic vasospasm and smooth muscle phenotype changes in arteries after SAH. Despite relief of angiographic vasospasm, phenotype changes persist in arterial walls after SAH.

The mechanisms involved in acute vasoconstriction and delayed cerebral vasospasm after subarachnoid hemorrhage (SAH) are different, and smooth muscle phenotype changes may contribute to delayed vasospasm. The phenotype of smooth muscle cells in the spastic cerebral arteries may change from a so-called contractile phenotype to a dedifferentiated synthetic phenotype that produces connective tissue and is associated with cell proliferation and migration.[1] In the present study, we have examined phenotypic changes in the spastic cerebral arteries after experimental SAH in a canine double-hemorrhage model. We hypothesized that cerebral smooth muscle cells change from the differentiated state to the dedifferentiated state during cerebral vasospasm. This phenotype change results in reduced contractile ability accompanied by extra production of connective tissues.

Methods

An established double-hemorrhage SAH canine model was used. Twenty-four dogs were divided randomly into four groups to be sacrificed on day 1,14, 21, and 28 after SAH. Angiography was performed on day 1 and on the days that the dogs were sacrificed. The spastic basilar arteries were collected, suspended in vitro under isometric tension, and challenged with high K+ or papaverine. The stiffness of the arterial wall was measured. The isometric tension study was performed as previously described using a specially designed apparatus.[2-3] Arterial rings were stretched stepwise to the same circumstance as was present in vivo as determined by angiography as previously reported.[3] Then the arterial ring was treated with papaverine, a nonselective vasodilator, to analyze the degree of myogenic tone. The amount of papaverine-sensitive tone was expressed as a percent of the developed stiffness. Morphological changes and collagen levels were examined by light microscopy and a dye-binding method, respectively. All data were expressed as mean ± standard error of the mean. Statistical significance of differences among groups was established according to Dunnett's multiple comparison tests after analysis of variance (ANOVA). Significance was established as $p < .05$.

Results

The angiographic diameter of the basilar artery on day 1 was 1.41 ± 0.05 mm (control = 100%, $n = 6$). The diameter of the basilar artery on each day was 45 ± 1% ($n = 6$) on day 14,75 ± 1% ($n = 6$) on day 21, and 91 ± 1% of control diameter ($n = 6$) on day 28. There was a statistically significant difference between arterial diameters on each day. The study in vitro of the spastic basilar arteries demonstrated increased stiffness of the arterial wall and dysfunctional contractile and relaxant ability at each time after SAH (Figs. 14–1 through 14–3). Light microscopy revealed round-shaped and shortened smooth muscle cells at days 14,21, and 28 after SAH. A remarkable increase in the thickness of the adventitia and migration of fibrous tissue were observed at days 14 to 21. Collagen concentration in the basilar artery after SAH was 304 ± 33,436 ± 31,297 ± 44, and 221 ± 24 µg/mg on days 1,14, 21, and 28 after SAH, respectively. The collagen concentration was significantly increased on day 14 after SAH compared with all other times ($p < .01$ vs day 1,21, and 28, ANOVA).

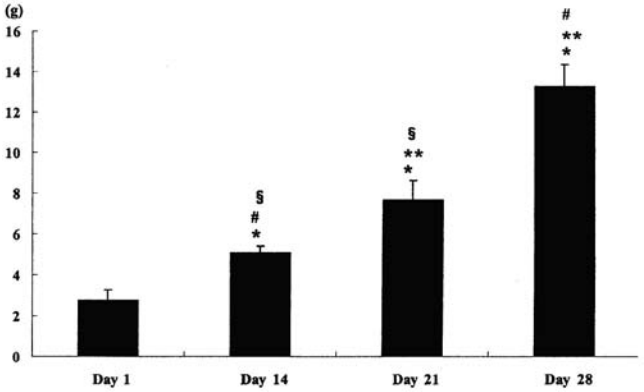


FIGURE 14–2 Bar graph showing tension of basilar arteries developed by increasing passive stretch under isometric tension. Bars represent means ±SEM. The basilar artery was stretched to the same circumference as that of the angiogram, according to the formula described previously (* $p < .01$ compared with day 1, ** $p < .01$ compared with day 14, # $p < .01$ compared with day 21, § $p < .01$ compared with day 28).[3]

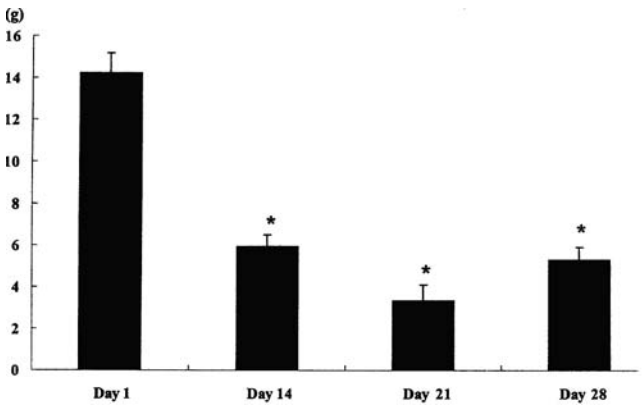


FIGURE 14–1 Bar graph showing contractile potency of the basilar artery elicited by a high K+ solution on day 1, day 14, day 21, or day 28 after SAH. Bars represent means ± SEM of the developed tension elicited by a high K+ solution (* $p < .01$ compared with day 1).

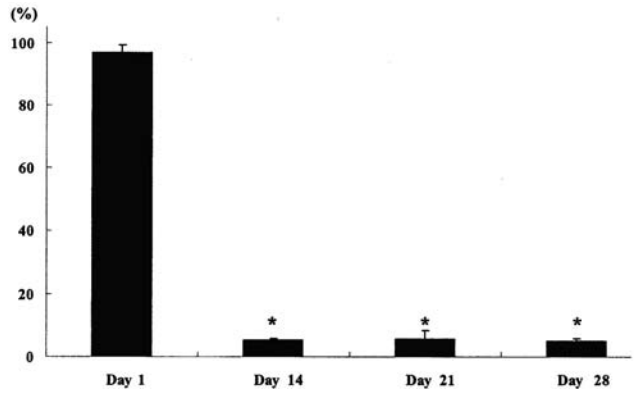


FIGURE 14–3 Bar graph (values are means ± SEM) showing effect of papaverine (0.1 mmol/L) on developed stiffness. The amount of papaverine-induced relaxation was expressed as a percent of the developed stiffness (* $p < .01$ compared with day 1).

Discussion

Enhanced arterial wall stiffness, reduced contractile potency, and diminished relaxant ability were observed at 2 to 4 weeks after SAH in the dog model. These observations indicate indirectly that smooth muscle cells switched phenotype from contractile to synthetic phenotype. The synthetic phenotype lacks arterial compliance, generates connective tissue, and increases vessel stiffness. Interestingly, even though angiography, morphology, connective tissue proliferation, and collagen content tend to return to normal levels at 28 days after SAH, the arterial compliance disturbance remains. Similar arterial compliance disorders were reported in the spastic arteries in other animal models.[4–6] We did not examine the phenotype of the smooth muscle cells directly in the present study. Additional work would need to be done to identify specific markers that are indicative of smooth muscle phenotype change in vasospastic arteries. In conclusion, despite relief of angiographic vasospasm within 14 days of experimental SAH, the present data suggest indirectly that a smooth muscle phenotype change continues for at least an additional 2 weeks.

REFERENCES

1. Borel CO, McKee A, Parra A, et al. Possible role for vascular cell proliferation in cerebral vasospasm after subarachnoid hemorrhage. Stroke 2003;34:427–433
2. Nakayama K, Obara K, Tanabe Y, Saito M, Ishikawa T, Nishizawa S. Interactive role of tyrosine kinase, protein kinase C, and Rho/Rho kinase systems in the mechanotransduction of vascular smooth muscles. Biorheology 2003;40:307–314
3. Nishizawa S, Peterson JW, Shimoyama I, Uemura K. Relation between protein kinase C and calmodulin systems in cerebrovascular contraction: investigation of the pathogenesis of vasospasm after subarachnoid hemorrhage. Neurosurgery 1992;31:711–716
4. Zhang J, Sima B, Johns LM, Macdonald RL. Time course of changes in arterial relaxation following subarachnoid hemorrhage in dogs. Neurol Res 1996;18:227–232
5. Kim P, Sundt TM Jr, Vanhoutte PM. Alterations of mechanical properties in canine basilar arteries after subarachnoid hemorrhage. J Neurosurg 1989;71:430–436
6. Macdonald RL, Zhang J, Sima B, Johns L. Papaverine-sensitive vasospasm and arterial contractility and compliance after subarachnoid hemorrhage in dogs. Neurosurgery 1995;37:962–967

15

Inflammation and Cerebral Vasospasm: New Perspectives

AARON S. DUMONT, M.D., TARKAN CALISANELLER, M.D., CHIH-LUNG LIN, M.D., NEAL F. KASSELL, M.D., KEVIN S. LEE, PH.D.

Abstract

Cerebral vasospasm persists as a leading cause of morbidity and mortality following subarachnoid hemorrhage (SAH). Lack of rational and efficacious therapy is due, at least in part, to an incomplete understanding of pathogenesis (particularly at the cellular and molecular levels). Increasing data support a pathogenic role for the inflammatory response accompanying SAH. The present review includes data that attempt to characterize cellular and molecular aspects of the inflammatory response following SAH. An intracisternal injection murine model of SAH was employed using a total of 200 C57Black/6J mice. Gene expression was assessed in control, sham-operated (saline injected into cisterna magna), and SAH-treated animals at 2 and 6 hours following hemorrhage induction using Affymetrix gene chips (Affymetrix, Santa Clara, CA). Complementary studies using genetically immunodeficient mice were also performed. Altered expression of *55* inflammatory related genes at one or two time points were demonstrated. Changes in gene expression were witnessed in key inflammatory genes including cytokines [e.g., interleukin-6 (IL-6) and IL-la, macrophage inflammatory protein-2 (MIP-2), and granulocyte-macrophage colony-stimulating factor (GM-CSF)]; adhesion molecules (e.g., E-selectin, P-selectin, and intercellular adhesion molecule-1); arachidonic acid metabolism enzymes (e.g., GriPGHS and 12-lipoxygenase); receptors for cytokines and prostaglandins including the IL-1 receptor type-1 and type-2; interferon receptor type-1 and prostaglandin F receptors; second messengers and inhibitors [e.g., supressor of cytokine signaling-2 (SOCS-2 and 3), interferon regulatory factor 1 (IRF-1), and extracellular regulated kinase 5 (ERK-5)]; oxidative stress mediators (heme oxygenase-1 and manganese superoxide dismutase); and extracellular matrix/vascular remodeling mediators [matrix metalloproteinases 3 and 12, tissue inhibitor of matrix metalloproteinase 2, and tissue factor pathway inhibitor 1 (TFPI-1)]. Cerebral vasospasm was significantly attenuated or abolished following SAH in immunodeficient mice (Severe Combined Immune Deficient and RAG-1-Recombination Activating Gene-1 –/– mice). These data show that SAH induces robust changes in the expression of many inflammation-related genes. Furthermore, immunologically deficient mice exhibit substantially less cerebral vasospasm than immunocompetent mice following SAH. Inflammation may be an integral mediator of the development and maintenance of cerebral vasospasm. Additional data in support of this hypothesis are reviewed.

Despite prodigious research efforts, the problem of cerebral vasospasm following subarachnoid hemorrhage (SAH) lingers. Recent work has provided a wealth of data concerning this disease entity, particularly in expansion of our understanding of the accompanying cellular and molecular events. The preexisting hypothesis that inflammation is the root cause of vasospasm is receiving renewed enthusiasm as data accrue.

Circumstantial Evidence

It is interesting to note that exposure of the subarachnoid space to blood and several different biological and physical substances produces similar phenomena. Antecedent inflammation of the subarachnoid space evokes arterial constriction. The injection of physical substances (such as talc or beads) results in inflammation and significant vasospasm while producing vessel morphological changes that mimic those seen following SAH.[1] Biological substances including craniopharyngioma and pituitary extract or tumor can also produce subarachnoid inflammation and subsequent vascular constriction. Furthermore, cerebral vasospasm is believed to complicate certain infectious processes of the subarachnoid space.[2] Collectively, these data provide indirect evidence for a critical role of inflammation in the genesis of cerebral vasospasm following SAH.

Direct Evidence

A growing body of research has directly examined the role of inflammatory processes in cerebral vasospasm following SAH through both clinical and laboratory studies. The role of leukocytes has been examined. The pathology of cerebral vasospasm involves an infiltrate of inflammatory cells.[3] Leukocytosis has been previously correlated with ischemic sequelae post-SAH, and a recent, well conducted study has established leukocytosis as an independent risk factor for cerebral vasospasm following aneurysmal SAH.[3,4] Kubota and colleagues have carefully examined the kinetics of leukocyte presence in the subarachnoid space using a rat SAH model.[5] They demonstrated a peak appearance of T cells and macrophages 2 days following SAH along with a peak in the CD4:CD8 T cell ratio at this time point. An initial cellular immune response followed by a humoral immune response with eicosanoid production was proposed. Recent work by Fassbender and colleagues has provided a fascinating connection between endothelin-1 (ET-1), a known key putative spasmogen following SAH, and inflammation.[6] Activated mononuclear cells synthesized and released ET-1 in parallel with known acute-phase reactants such as interleukin-1ß (IL-1ß), IL-6, and tumor necrosis factor-a.

The process of leukocyte migration to areas of active inflammation has also been extensively examined in the context of SAH. Altered levels of soluble adhesion levels and cytokines critical in the recruitment and migration of leukocytes have been noted in the cerebrospinal fluid (CSF) and plasma following SAH.[3] Moreover, therapies targeted at minimizing leukocyte recruitment, adhesion, and migration such as monoclonal antibodies against intercellular adhesion molecule-1 significantly diminish cerebral vasospasm.[7]

Additionally, transcription factors and enzymes regulating expression of these molecules and other constituents of the inflammatory response (such as the ubiquitous transcription factor nuclear factor-kappa B (NF-KB) and the nuclear enzyme poly(ADP-ribose) polymerase (PARP) have demonstrated importance in the development of cerebral vasospasm.[3]

Immunoglobulins and complement may also play a role in cerebral vasospasm pursuant to SAH. Increased levels of immunoglobulins and complement have been found in the serum and vessel walls in the context of SAH. Complement activation appears to accelerate the liberation of spasmogenic substances by promoting erythrocyte lysis, and complement depletion reduces vasospasm.[3-8]

Murine Model of Subarachnoid Hemorrhage

Increased interest in the cellular and molecular basis of disease processes has made the use of mice invaluable. The knowledge of their genome, the widespread availability of molecular biological tools for this species, and the ability to create genetically manipulated animals (so-called knockout and transgenic mice) make them a logical choice for dissecting cellular and molecular contributions to entities such as cerebral vasospasm.

We have recently developed and reported a murine model of SAH utilizing a direct intracisternal (into the cisterna magna) injection of autologous blood that is simple, effective, reproducible, and inexpensive with a low mortality rate.[9] This model has afforded opportunities for subsequent studies of large-scale gene expression and genetically manipulated animals.

Gene Expression Studies and Genetically Manipulated Animals

Several laboratories have examined gene expression using various methods and animal species.[3] Studies using rat, canine, and primate models have revealed changes in expression of numerous genes including many inflammation-related genes such as intercellular

adhesion molecule-1, IL-a, IL-6, IL-8, and monocyte chemotactic protein-1 among others.[3] Work from our laboratory has similarly demonstrated significant alteration in the expression of many inflammation-related genes using a murine model of SAH and gene chip technology (unpublished observations).

As mentioned, genetically manipulated animals have been useful in examining the contribution(s) of individual factors or combinations thereof. Preliminary experiments from our laboratory utilizing genetically manipulated animals (adhesion molecule knock-out and immunodeficient animals) have provided promising data emphasizing the critical importance of inflammation in cerebral vasospasm following SAH (unpublished observations).

Conclusion

Cerebral vasospasm following SAH remains incompletely understood and ineffectively prevented and treated clinically. Accumulating data implicating a critical role of inflammation in the development and maintenance of cerebral vasospasm have provided new impetus for directed future mechanistic and therapeutic studies. Further delineation of the underlying cellular and molecular basis of this unique disease will facilitate the development of rational targeted therapy (pharmacological and/or gene based). Such work will hopefully bring the intensive and extensive efforts of the last 5 decades to fruition as cerebral vasospasm is carried through to its ultimate solution.

REFERENCES

1. Peterson JW, Kwun B, Hackett JD, Zervas NT. The role of inflammation in experimental cerebral vasospasm. J Neurosurg 1990; 72:767–774
2. Ries S, Schminke U, Fassbender K, Daffertshofer M, Steinke W, Hennerici M. Cerebrovascular involvement in the acute phase of bacterial meningitis. J Neurol 1997;244:51–55
3. Dumont AS, Dumont RJ, Chow MM, et al. Cerebral vasospasm after subarachnoid hemorrhage: putative role of inflammation. Neurosurgery 2003;53:123–135
4. McGirt MJ, Mavropoulos JC, McGirt LY, et al. Leukocytosis as an independent risk factor for cerebral vasospasm following aneurysmal subarachnoid hemorrhage. J Neurosurg 2003;98: 1222–1226
5. Kubota T, Handa Y, Tsuchida A, Kaneko M, Kobayashi H, Kubota T. The kinetics of lymphocyte subsets and macrophages in subarachnoid space after subarachnoid hemorrhage in rats. Stroke 1993;24:1993–2001
6. Fassbender K, Hodapp B, Rossol S, et al. Endothelin-1 in subarachnoid hemorrhage: an acute-phase reactant produced by cerebrospinal fluid leukocytes. Stroke 2000;31:2971–2975
7. Bavbek M, Polin R, Kwan A, Arthur AS, Kassell NF, Lee KS. Monoclonal antibodies against ICAM-1 and CD18 attenuate cerebral vasospasm after experimental subarachnoid hemorrhage in rabbits. Stroke 1998;29:1930–1936
8. Peterson JW, Searle R, Mandy FF, et al. Immunological reaction against the aging human subarachnoid erythrocyte. J Neurosurg 1989;71:718–726
9. Lin CL, Calisaneller T, Ukita N, Dumont AS, Kassell NF, Lee KS. A murine model of subarachnoid-induced cerebral vasospasm. J Neurosci Methods 2003;123:89–97

SECTION III

Experimental—Endothelium

16

Pathophysiology of Delayed Vasospasm After SAH: New Hypothesis and Implications for Treatment

RYSZARD M. PLUTA, M.D., PH.D.

Abstract

Despite years of research and hundreds of successful experimental treatments, delayed vasospasm remains a feared untreatable complication of subarachnoid hemorrhage (SAH) after rupture of an intracranial aneurysm. This chapter presents a new hypothesis for vasospasm development based on the research that has been done in the Vascular Laboratory of Surgical Neurology Branch of the National Institutes of Neurological Disorders and Stroke. It is well established that levels of ferrous hemoglobin slowly released from erythrocytes after SAH in the vicinity of the artery harboring the aneurysm are correlated with the temporal development of vasospasm. Oxyhemoglobin is neurotoxic and destroys neurons in the adventitia of conductive arteries, such as nitric oxide (NO)-releasing neurons from the sphenopalatine ganglion that under normal conditions are responsible for vasorelaxation. When oxyhemoglobin release from a subarachnoid clot peaks approximately 7 days after SAH, neuronal NO synthase (nNOS)-expressing neurons disappear from the arterial adventitia. This diminishes NO availability in the vessel wall and leads to the initial vasoconstriction (Phase I of delayed vasospasm). However, such a narrowing of the arterial wall is a powerful stimulator of endothelial NOS (eNOS) activity via increased shear stress. Increased endothelial production of NO should then counteract decreased NO production due to lack of nNOS. However, at the same time in the perivascular space, hemoglobin is further metabolized to bilirubin oxidization products (BOXs) that can penetrate the arterial wall and increase levels of asymmetric dimethyl arginine (ADMA), an endogenous inhibitor of eNOS, via methylation of L-arginine and/or inhibition of ADMA hydrolysis. One or both of these mechanisms can increase the levels of ADMA, leading to further decrease of NO availability and sustenance of vasospasm (Phase II). Fortunately, further oxidation and elimination of BOXs increases ADMA hydrolysis by asymmetric dimethyl arginine dimethylamino hydrolase (DDAH II). This leads to the increase of NO production by eNOS, recovery of endothelial dilatory activity, and resolution of vasospasm (Phase III). This three-stage hypothesis of delayed cerebral vasospasm may yield a novel treatment for vasospasm based on actual pathophysiological events rather than on the dilatory activity of the agents used. Directly after SAH until day 3, the key treatment is to remove the clot and bloody cerebrospinal fluid to decrease levels of neurotoxic hemoglobin. This should prevent death of noxinergic neurons in the adventitia of the arteries and block an initial spasm of the arteries as well as decrease the

availability of oxyhemoglobin that can be metabolized to BOXs. However, it is unlikely that all the blood can be removed. Thus, pharmacological prevention of activation of ADMA production by BOXs should be started no later than on day 3 following SAH. If despite all these efforts vasospasm develops, NO should be administered intra-arterially or locally, and its vasodilatory effect should be supported by inhibition of ADMA leading to increased production of endogenous NO via activation of eNOS.

Despite years of research and many successful experimental treatments, delayed vasospasm remains a feared and untreatable complication of subarachnoid hemorrhage (SAH) following rupture of an intracranial aneurysm. This study presents a new hypothesis of vasospasm development that is based on our research.

In cerebral vessels, nitric oxide (NO), also known as endothelium-derived relaxing factor, regulates vascular tone and arterial dilatation in response to increased pCO_2.[1–3] In brain arteries, NO is produced by endothelial and neuronal nitric oxide synthases (NOS).[3,4] NO acts via activation of guanylate cyclase through nitrosylation of the heme moiety of NOS, leading to increased cyclic guanosine monophosphate production, hyperpolarization of the smooth muscle cell membrane by opening of K+ channels, and modulation of Ca^{2+} channels (Fig. 16–1).[5] Following SAH, such regulation of vascular tone can be disturbed[2,6] by the high affinity of hemoglobin for NO ("sink effect"),[7] slowly released from erythrocytes in the vicinity of the arterial wall (Fig. 16–2).[8] However, the thickness of the conductive artery wall coupled with the limited penetration of oxyhemoglobin through the arterial wall[9] precludes the "sink effect" from having a significant role in scavenging NO after SAH. This observation is supported by decreased nitrite/nitrate levels in cerebrospinal fluid (CSF) after SAH in both experimental and clinical settings, suggesting that development of vasospasm is due to decreased NO production, not to the scavenging of NO by hemoglobin.

Another suggested mechanism by which NO may evoke vasospasm is a direct cytotoxic effect of peroxynitrite. Peroxynitrite is a by-product of NO produced by activation of inducible NOS in macrophages on smooth muscle cells.[9,10] However, the low levels of inducible NOS in the arterial wall[10] and the unlikely biochemical transformation of NO into peroxynitrite in the arterial wall[11] limit the possible role of peroxynitrite in evoking vasospasm. On the other hand, oxyhemoglobin, which is directly or indirectly responsible for development of vasospasm,[12] is also known for its neurotoxic effect.[13,14] In fact, it has been reported that when oxyhemoglobin levels peak at approximately day 7 after an SAH,[8] neuronal NO synthase (nNOS)–expressing (nitroxic, neuronal NOS-containing) neurons disappear from the arterial adventitia (Fig. 16–2).[4] These neurons arising from the sphenopalatine ganglion are, under normal conditions, responsible for vasorelaxation.[3] The lack of nNOS diminishes NO availability in the vessel wall and leads to the initial vasoconstriction (Phase I, initiation of delayed vasospasm). Such a narrowing of the arterial wall is a powerful stimulator of endothelial NOS (eNOS)[15] because of increased shear stress.[16] Consequently, an increased NO production by stimulated eNOS should counteract a decreased NO availability due to a long-lasting lack of nNOS activity in the adven-

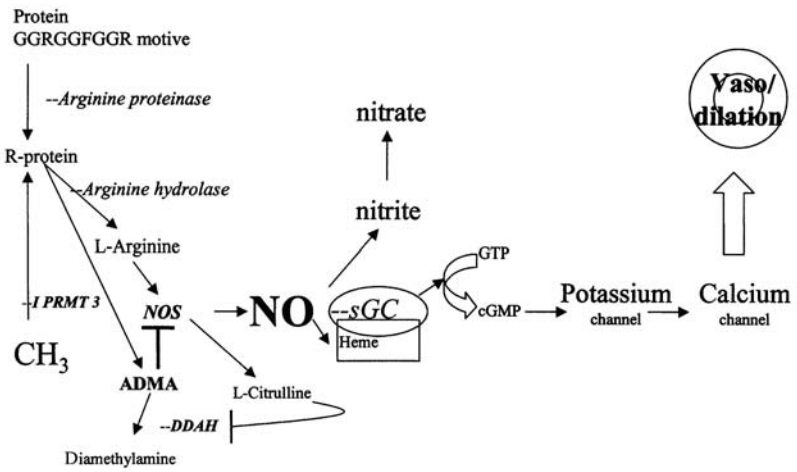


FIGURE 16–1 Schematic represents nitric oxide (NO)-related signal transduction and inhibition of endothelial nitric oxide synthase (eNOS) by asymmetric dimethyl arginine (ADMA; description in the text).

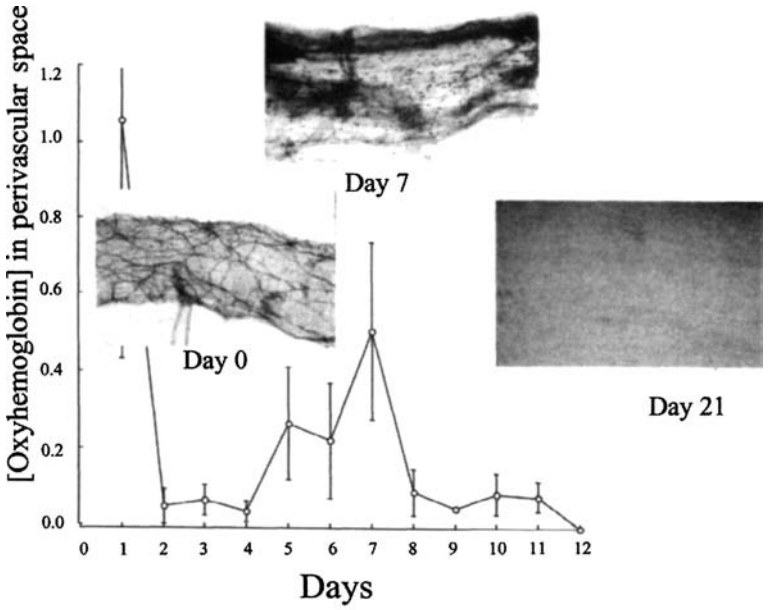


FIGURE 16–2 The graph depicts neuronal nitric oxide synthase (nNOS) expression in the arterial wall and corresponding levels of oxyhemoglobin in the vicinity of the conductive artery after subarachnoid hemorrhage (SAH) during development and resolution of vasospasm.

titia, leading to quick resolution of vasospasm.[4] However, the persistence of vasospasm, decreased cyclic guanosine monophosphate levels in the arterial wall, and decreased levels of nitrites with preserved expression of eNOS[15] suggest the existence of an NO-related endothelial dysfunction that decreases NO production. Such an endothelial dysfunction has been demonstrated in arthrosclerosis, renal insufficiency, and hypertension.[13,17]

Data from experiments conducted in vivo and presented elsewhere in this volume confirm the development of NO-related endothelial dysfunction at the time of vasospasm after SAH.[18] The cellular mechanism of endothelial dysfunction is unclear, but it has been suggested that it depends on the increased presence of asymmetric dimethyl arginine (ADMA), an endogenous inhibitor of eNOS (see Fig. 16–1).[19] Therefore, we hypothesized that the increased ADMA levels may be responsible for endothelial dysfunction leading to the next phase of vasospasm. Work by Jung et al described elsewhere in this book (chapter 21) confirms that ADMA is a source of such dysfunction during vasospasm following SAH.[20] Thus, after a decreased NO production by initial elimination of nNOS ("first hit") by oxyhemoglobin, the increased ADMA levels in the perivascular space lead to a further decrease of NO availability ("second hit"; Phase II, sustenance of delayed vasospasm).

To better understand the pathophysiology of this phase of vasospasm, we sought to establish the source and cellular mechanism of ADMA production and the possibility of its pharmacological inhibition in experiments in vitro that mimic SAH. Endothelial cells were exposed to oxyhemoglobin and/or bilirubin oxidation products (BOXs) recently purported agents associated with vasospasm in clinical and experimental settings[21] and ADMA levels were measured in the media and the cells. This experiment revealed that BOXs but not oxyhemoglobin increased ADMA levels in human umbilical vein endothelial cells and the media of the cultures. Furthermore, probucol (Vyrex, La Jolla, CA, USA), an antihypercholesterolemic drug,[22] inhibited the increase of ADMA levels evoked by BOXs. For this reason, we are using probucol in a randomized, double-blind, placebo-controlled experiment to prevent development of vasospasm in a primate model of SAH. In the last phase of vasospasm, oxidation and elimination of BOXs reduce ADMA levels in the CSF, resulting in increased NO production by eNOS and recovery of endothelial dilatory activity (Phase III, resolution of delayed vasospasm) despite the persistent absence of nNOS activity (see Fig. 16–2).[4]

These results support a hypothesis that delayed vasospasm following SAH develops in three phases (Fig. 16–3). The presented mechanisms of development and sustenance of vasospasm may yield a novel treatment for this life-threatening complication of SAH. The initial treatment directly after SAH until day 3 is to remove the clot and bloody CSF to decrease levels of neurotoxic oxyhemoglobin in the vicinity of conductive vessels. This should prevent death of noxinergic neurons in the adventitia of the arteries and block an initial spasm of the arteries as well as decrease the availability of oxyhemoglobin that can be metabolized to BOXs. However, it is unlikely that all the blood can be removed. Thus, pharmacological prevention of activation of ADMA production by BOXs should be started no later than on day 3 following SAH.

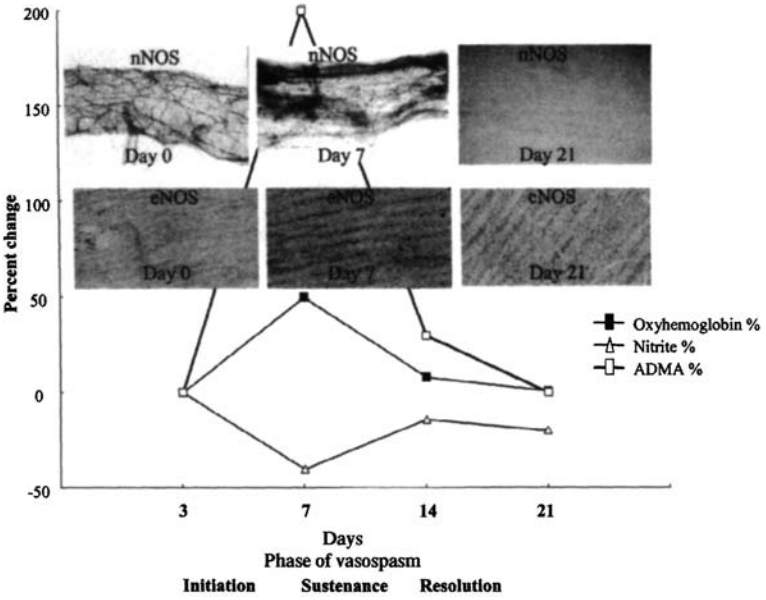


FIGURE 16–3 Schematic describes the three stages of vasospasm after subarachnoid hemorrhage (SAH). First, oxyhemoglobin destroys the neuronal nitric oxide synthase (nNOS)-containing nerves in the adventitia, and this decreases availability of nitric oxide (NO) in the vessel wall confirmed by a decrease of nitrite levels evoking vasoconstriction (Phase I, initialization of vasospasm). Under normal conditions this effect should be limited by activation of endothelial nitric oxide synthase (eNOS) via a significant increase of shear stress due to decreased vessel diameter. However, bilirubin oxidation products (BOXs) cleaved from the hemoglobin-degradation product bilirubin increase the levels of asymmetric dimethyl arginine (ADMA), probably by inhibition of asymmetric dimethyl arginine dimethylamino hydrolase (DDAH), evoke secondary inhibition of NO production and prolongation of vasospasm, and further increase the inflammatory response via an increase of cytokines (Phase II, sustenance of vasospasm). Then, a gradual elimination of hemoglobin and BOXs leads to decreased ADMA levels and increased levels of nitrite in the cerebrospinal fluid (CSF) and resolution of vasospasm (Phase III, resolution of vasospasm).

REFERENCES

1. Lavi S, Egbaraya R, Lavi R, et al. Role of nitric oxide in the regulation of cerebral blood flow in humans. Circulation 2003;107:1901–1905
2. Thompson BG, Pluta RM, Girton M, et al. Nitric oxide mediation of chemoregulation but not autoregulation of cerebral blood flow in primates. J Neurosurg 1996;84:71–78
3. Toda N, Tanaka T, Ayajiki K, et al. Cerebral vasodilatation induced by stimulation of the sphenopalatine ganglion and greater petrosal nerve in anesthetized monkeys. Neuroscience 2000;96:393–398
4. Pluta R, Thompson B, Dawson T, et al. Loss of nitric oxide synthase immunoreactivity in cerebral vasospasm. J Neurosurg 1996;84:648–654
5. Archer S, Huang J, Hampl V, et al. Nitric oxide and cGMP cause vasorelaxation by cGMP-kinase-dependent activation of charybdotoxin-sensitive K channel. Proc Natl Acad Sci USA 1994;91:7583–7587
6. Iuliano B, Pluta R, Jung C, et al. Endothelial dysfunction in a primate model of cerebral vasospasm. J Neurosurg. 2004;100: 287–294
7. Ignarro L. Biosynthesis and metabolism of endothelium-derived nitric oxide. Annu Rev Pharmacol Toxicol 1990;30:535–560
8. Pluta R, Afshar J, Boock R, et al. Temporal changes in perivascular concentrations of oxyhemoglobin, deoxyhemoglobin and, methemoglobin after subarachnoid hemorrhage. J Neurosurg 1998;88:557–561
9. Foley P, Kassell N, Hudson S, et al. Hemoglobin penetration in the wall of the rabbit basilar artery after subarachnoid hemorrhage and intracisternal hemoglobin injection. Acta Neurochir (Wien) 1993;123:82–86
10. Suzuki S, Kassell N, Lee K. Hemin activation of an inducible isoform of nitric oxide synthase in vascular smooth-muscle cells. J Neurosurg 1995;83:862–866
11. Espey MG, Thomas DD, Miranda KM, et al. Focusing of nitric oxide mediated nitrosation and oxidative nitrosylation as a consequence of reaction with superoxide. Proc Natl Acad Sci USA 2002;99:11127–11132
12. Macdonald R, Weir B. A review of hemoglobin and the pathogenesis of cerebral vasospasm. Stroke 1991;22:971–982
13. Cooke J. Does ADMA cause endothelial dysfunction? Arterioscler Thromb Vasc Biol 2000;20:2032–2037
14. Wang X, Mori T, Sumii T, et al. Hemoglobin-induced cytotoxicity in rat cerebral cortical neurons: caspase activation and oxidative stress. Stroke 2002;33:1882–1888
15. Kasuya H, Weir B, Nakane M, et al. Nitric oxide synthase and guanylate cyclase levels in canine basilar artery after subarachnoid hemorrhage. J Neurosurg 1995;82:250–255
16. Buga G, Gold M, Fukuto J, et al. Shear stress-induced release of nitric oxide from endothelial cells grown on beads. Hypertension 1991;17:187–193
17. Vallance P, Chan N. Endothelial dysfunction and nitric oxide: clinical relevance. Heart 2001;85:342–350
18. Iuliano B, Pluta RM, Jung C, Oldfield EH. Endothelial dysfunction in a primate model of cerebral vasospasm. In: Macdonald RL, ed. Cerebral Vasospasm: Advances in Research and Treatment. New York: Thieme Medical Publishers; 2004
19. Vallance P, Leone A, Calver A, et al. Endogenous dimethylarginine as an inhibitor of nitric oxide synthesis. J Cardiovasc Pharmacol 1992;20(suppl 12):S60–S62
20. Jung C, Iuliano B, Harvey-White J, et al. CSF levels of ADMA, an endogenous inhibitor of nitric oxide synthase, are associated with cerebral vasospasm after SAH. In: Macdonald RL, ed. Cerebral Vasospasm: Advances in Research and Treatment. New York: Thieme Medical Publishers; 2004
21. Clark J, Reilly M, Sharp F. Oxidation of bilirubin produces compounds that cause prolonged vasospasm of rat cerebral vessels: a contributor to subarachnoid hemorrhage-induced vasospasm. J Cereb Blood Flow Metab 2002;22:472–478
22. Jiang J-L, L N-S, Deng H-W. Probucol preserves endothelial function by reduction of the endogenous nitric oxide synthase inhibitor level. Br J Pharmacol 2002;135:1175–1182

17

Endothelin B Receptor Null Mutation Prevents Subarachnoid Hemorrhage-Induced Cerebral Vasospasm in the Rat In Vivo

JAVIER FANDINO, M.D., ROBERT M. RAPOPORT, PH.D., CHERYL E. GERIEPY, M.D., ROLF W. SEILER, M.D., MASASHI YANAGISAWA, PH.D., MARIO ZUCCARELLO, M.D.

Abstract

Support for endothelin (ET)-1 mediation of subarachnoid hemorrhage (SAH)-induced vasospasm is partly derived from ET_A receptor antagonist inhibition of spasm. While these findings suggest the lack of ET_B receptor involvement, this possibility remains. To investigate this possibility, we utilized the spotting lethal (sl) rat, which carries a naturally occurring deletion in the ET_B receptor gene that prevents functional receptor expression, and also the tissue-specific ET_B transgene that allows normal enteric nervous system development. Vertebrobasilar angiography was performed in sl and +/+ (wild type) rats to determine baseline arterial diameters. This was followed by injection of autologous arterial blood into the cisterna magna. Follow-up angiography was performed 2 days post-SAH. Basilar artery spasm magnitude was calculated as percent change (mean ± SEM) by comparison of pre- and post-SAH angiograms. SAH was associated with 13.1 ± 0.9% spasm in +/+ rats (n = 3), which was similar to the magnitude of vasospasm in Wistar rats (14.2 ± 1.0%, n = 14). In contrast, SAH did not elicit spasm in sl/+ (0.2 ± 0.5%, n = 5) and sl/sl rats (-3.8 ± 1.2%, n = 6). Control saline injections did not induce spasm. Mortality following SAH and angiography in +/+, sl/+, and sl/sl rats was high at *75,* 72, and *55%,* respectively, and was 25% in Wistar rats. These results demonstrate that ET_B receptors are essential for SAH-induced spasm of the rat basilar artery.

Experimental evidence suggests that endothelin (ET) plays an important role in subarachnoid hemorrhage (SAH)-induced cerebral vasospasm. ET-1 acts by at least three different receptor subtypes: an ET_A and two different ET_B receptor subtypes (ET_{B1} and ET_{B2}). The ET_A receptor has high affinity for ET-1 and ET-2 and less affinity for ET-3. The ET_{B1} receptor is present in vascular endothelial cells and mediates the endothelium-dependent vasodilatative action of ET. The ET_{B2} receptor subtype is present in smooth muscle cells and is associated with vasoconstriction. ET_A and ET_B receptors are involved in the development of SAH-induced vasospasm. A dynamic relation between ET_A and ET_{B2} receptors has been proposed. The purpose of this study was to investigate the involvement of ET_B receptor in the development of SAH-induced vasospasm. For these purposes, we utilized a spotting lethal (sl) rat that carries a naturally occurring deletion in the ET_{B1} and ET_{B2} receptor gene that prevents functional receptor expression.

Materials and Methods

Mutant Rats

Procedures were approved by the Institutional Animal Care and Use Committee. Thirty Wistar rats and a total of 65 transgenic rats (weight, 250–450 g) were used. Transgenic rats carry a naturally occurring deletion in the ET_B receptor gene that prevents functional receptor expression [spotting lethal (sl) rats]. Rats homozygous for this autosomal recessive mutation (sl) exhibit coat color spotting and congenital intestinal aganglionosis. These deficits result from failure of the neural crest–derived epidermal melanoblasts and enteric nervous system precursors to completely colonize the skin and intestine, respectively. Using the human dopamine-ß-hydroxylase promoter to direct transgenic expression of the ET_B receptor gene to colonize enteric nervous system precursors in the sl/sl rat, the intestinal defect could be prevented. Aganglionosis rats were crossed with a Sprague-Dawley rat carrying a dopamine-ß-hydroxylase-ET_B receptor transgene. The dopamine-ß-hydroxylase-ET_B receptor transgenic compensation prevents the death of the rats from intestinal obstruction before or shortly after weaning and allows normal development. The rats can be classified into sl /sl transgene hemizygous, sl/sl transgene homozygous, sl/+ transgene heterozygous, and wild type (+/+). Both heterozygous sl/+ and congenic + / + rats lack pigment in approximately 50% of their coat. They are otherwise healthy. Homozygous sl/sl rats have dark eyes and white coat. Occasionally, they have small pigmented areas on their head and hips (Fig. 17–1).[1] We used phenotype to distinguish the sl/sl from the +/sl rats. The +/+ rats were obtained from genetically analyzed breeders by mating of + /sl with +/sl. In a few animals with unclear phenotype, phenotype was determined by genotyping using polymerase chain reaction and Northern blot analyses of the tails. In our study, a total of 65 transgenic rats were used (sl/sl: n = 18; sl/+: n = 22; and + / + :n = 25).

FIGURE 17–1 Phenotype of transgenic rats. Both heterozygous sl/+ and congenic +/+ (wild type) rats lack pigment in ~50% of their coat. Homozygous sl/sl rats have dark eyes and white coat and occasionally have small pigmented areas in their head and hips.

Cerebral Angiography

Anesthesia was induced by intraperitoneal injection of pentobarbital sodium (50 mg/kg) or ketamine hydrochloride (100 mg/kg intramuscularly) and xylazine (10 mg/kg intramuscularly). In addition, buprenorphine hydrochloride (0.2 mg/kg) was injected subcutaneously to alleviate pain once the animals awoke from anesthesia. Animals were allowed to breath spontaneously in a supine position. The ventral midcervical region was shaved, and a skin incision was made for microsurgical catheterization of the internal carotid artery after ligation of the external carotid artery. Iodine radiocontrast medium was injected at a rate of 0.5 mL/sec for 2 seconds, and images (5 degrees left anterior oblique angle) of the vertebrobasilar system were obtained at 2 per second for 14 seconds with a rapid sequential angiographic technique. Digital subtraction analysis was performed with a small focal spot at 60 kV and 0.8 mA (DFP 2000A, Toshiba Medical Systems Toshiba America Medical Systems, Tustin, CA). Angiograms were performed on day 0 immediately before SAH and on day 2 after SAH. The diameter of the basilar artery was measured by image analysis. Images were collected by placement of angiograms on a light box fixed with a video camera. Three measurements were made at levels just below the basilar–posterior cerebral artery junction, just above the basilar–vertebral artery junction and midway between these locations. These nine values were averaged and constriction was expressed as a percent of the basilar artery diameter on the follow-up angiogram relative to the angiogram on day 0 prior to SAH.

Experimental SAH

After performing the initial angiogram, the inguinal region was shaved and prepared in a sterile manner.

After exposing the right femoral artery and preparing it for cannulation to obtain arterial blood, the animals were positioned in a prone position and immobilized in a stereotactic frame. A midline incision on the dorsal surface of the neck was made from the cranial vertex to the lower cervical spine. The suboccipital and nuchal muscles were divided bilaterally to expose the atlantooccipital membrane. The cisterna magna was punctured with a 25-gauge needle, and 0.1 mL cerebrospinal fluid (CSF) was aspirated. A total of 0.2 mL autologous arterial blood was withdrawn from the femoral artery and injected into the subarachnoid space. Finally, the wound was closed and the head was positioned downward for 5 minutes to allow distribution of the injected blood in the basal cisterns. Angiography was repeated 2 days after SAH using the technique just described, and animals were euthanized by injection of an overdose of pentobarbital.

Results

SAH-Induced Vasospasm

Fifty-three experiments were completed. SAH was associated with 13.1 ± 0.9% vasospasm in +/+ (wild type) rats (n = 3), which was similar to the magnitude of spasm in Wistar rats (14.2 ± 1.0%, n = 14). In contrast, SAH did not elicit spasm in sl/+ (0.2 ± 0.5%, n = 5) and sl/sl rats (–3.8 ± 1.2%, n = 6, p < .001, Figs. 17–2 and 17–3). Control saline injections did not induce vasospasm (Wistar rats: *-5.7* ± 2.6% change in basilar artery diameter, n = 9; sl/sl rats: -0.75 ± 0.75%, n = 2; sl/+ rats: -5 ± 0.6%, n = 3).

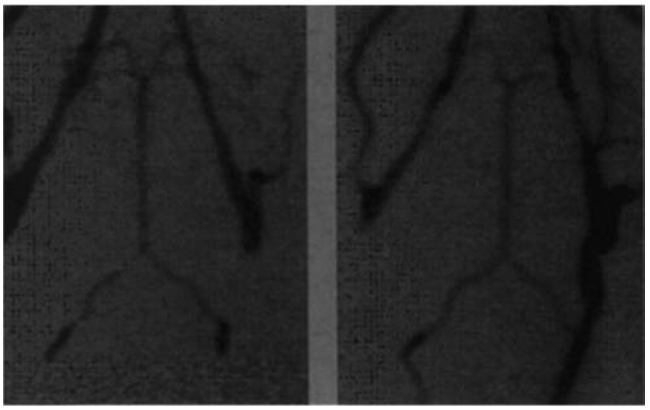


FIGURE 17–2 Photographs of angiography of rat vertebral basilar arterial system. Angiography on day 0 before subarachnoid hemorrhage (SAH) (left) and on day 2 after SAH (right) shows failure of SAH to induce vasospasm in a rat carrying a deletion in the ET_B receptor gene (transgenic sl/sl male rat).

Mortality

A significantly higher mortality following SAH and angiography was observed in the transgenic rats (73%) compared with the Wistar rats (23%). Within the transgenic rats, the higher mortality was observed in the wild type (+/+) group (88%), followed by the +/sl and the sl/sl groups (72% and 55%, respectively). The highest mortality in the transgenic rats, especially in the +/+ group, was observed immediately after the first angiogram or SAH. There was no explanation for the high mortality in this group of animals.

Discussion

The present study represents the first demonstration that the presence of ET_B receptors in the cerebral arteries is important for the development of SAH-induced vasospasm. In support of this is the lack of angiographic evidence of vasospasm of the basilar artery after SAH in sl/sl transgene hemizygous rats that carry a naturally occurring deletion in the ET_B receptor gene that prevents functional receptor expression (—3.8 ± 1.2%). The transgenic sl/+ rats, which have a phenotype and in which a low level of expression of ET_B receptor gene therefore might be speculated, also failed to develop SAH-induced vasospasm (0.2 ± 0.5%). In comparison to these two groups, a 13.1 ± 0.9% degree of vasospasm in +/+ (wild type) rats could be documented (p < .0001). Although the current concept of the complex etiology of SAH-induced vasospasm is based on the presumption of a multifactoral origin, there is considerable evidence to suggest that ET-1 plays an essential role in the pathogenesis of vasospasm. Since the development of synthetic ET_A, ET_B, and $ET_{A/B}$ receptor antagonists, dynamic mechanisms between receptors could be demonstrated. Recently, we proposed that the mechanism of ET_B receptor antagonist attenuation of the spasm is largely through blockade of ET_B receptor–mediated ET-1-induced ET-1 release, rather than blockade of ET_B receptor-mediated ET-1-induced constriction.[2] Considering the results of this study it can be speculated that ET_B functional receptor expression is indispensable for the development of ET-1-induced vasospasm in the rat SAH model.

Conclusion

This study suggests that ET_B receptors are important in SAH-induced vasospasm, possibly via ET_{B1}-mediated ET-1 release. More direct studies are required to further test this hypothesis.

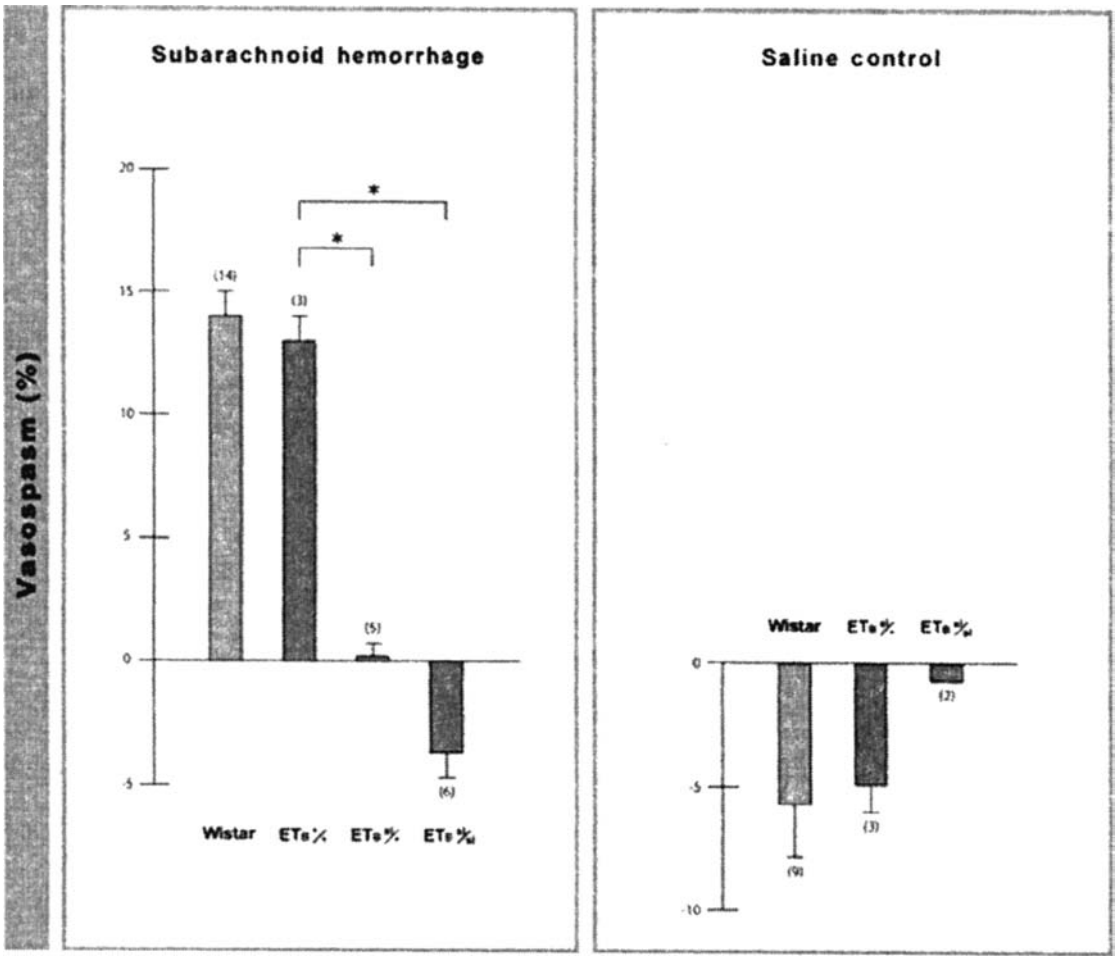


FIGURE 17–3 Bar graphs showing changes in diameter of the rat basilar artery after injection of blood (left) or saline (right) in the cisterna magna. Subarachnoid hemorrhage (SAH) was associated with vasospasm in +/+ (wild type, ET_B +/+) rats, which was similar to the magnitude of vasospasm in Wistar rats. In contrast, SAH did not elicit spasm in sl/+ (ET_B sl/+) and sl/sl (ET_B sl/sl) rats. Control injections of saline did not induce spasm (* $p < .0001$ compared with day 0 within groups).

REFERENCES

1. Gariepy CE, Richardson JA, Hammer RE, Yanagisawa M. Transgenic expression of the endothelin-B receptor prevents congenital intestinal aganglionosis in a rat model of Hirschsprung disease. J Clin Invest 1998;102:1092–1101
2. Zuccarello M, Romano A, Rapoport RM. Endothelin B receptor antagonists attenuate subarachnoid hemorrhage–induced cerebral vasospasm. Stroke 1998;29:1924–1929

18

Experimental SAH Alters Endothelin Receptor Phenotype in Rat Cerebral Arteries

JACOB HANSEN-SCHWARTZ, M.D., PH.D., NATALIE LØVLAND HOEL, B.S., MINGFANG ZHOU, M.D., CANG-BAO XU, M.D., PH.D., NIELS-AAGE SVENDGAARD, M.D., PH.D., LARS EDVINSSON, M.D., PH.D.

Abstract

Organ culture of rat cerebral arteries is associated with alteration in the vascular endothelin (ET) receptor population. We investigated if such changes also occur in cerebral arteries in a rat subarachnoid hemorrhage (SAH) model. SAH was induced by injecting 250 µL of blood into the prechiasmatic cistern. After 2 days the middle cerebral, basilar, and posterior communicating arteries were harvested. Pharmacological studies were performed in vitro, and levels of messenger ribonucleic acid (mRNA) were quantified using real-time reverse transcriptase polymerase chain reaction (RT-PCR). Middle cerebral and basilar arteries from rats with SAH exhibited an enhanced biphasic response to ET-1. The pEC_{50} of the high affinity phase was ~12 versus ~8.5 in sham operated animals. At concentrations of ET-1 equal to the physiological concentration of ET-1 in the plasma ($\sim 10^{-9}$ mol/L), a contraction of 50 to 75% of the contraction obtained through stimulation with K+, 60 mmol/L, was observed. Quantitative mRNA studies in the same arteries showed a significant increase in the number of copies of ET_B but not ET_A receptor mRNA. Evidence of a functional ET_B receptor was provided through antagonist studies. The posterior communicating artery did not undergo significant changes. The altered receptor profile observed may represent the end stage in the chain of events leading from SAH to the actual spasm of the artery. The pharmacological data concerning the ET_B receptor suggest a not hitherto appreciated complex interaction between a normally present ET_A receptor and an upregulated ET_B receptor.

Inspired by the discoveries that organ culture of rat cerebral arteries as well as temporary occlusion of the middle cerebral artery in the rat leads to the appearance of contractile endothelin B (ET_B) receptors (in fresh cerebral arteries only a relaxant response is observed),[1-2] and that the organ culture procedure also leads to increased sensitivity to ET, we set out to investigate the effect of experimental subarachnoid hemorrhage (SAH) in rats on the expression and function of ET receptors in cerebral arteries. For investigation we selected the middle cerebral, posterior communicating, and basilar arteries.

Methods

Rat Subarachnoid Hemorrhage Model

Procedures were conducted as described previously.[3] Briefly, male Sprague-Dawley rats (350–400 g, M & B, Denmark) were anesthetized (both induction and maintenance) with halothane. Catheters were placed to monitor arterial blood pressure, intracranial pressure, and cerebral blood flow. For the induction of SAH, a 27-gauge, blunt-ended cannula with a side hole was introduced stereotactically 7.5 mm anterior to the bregma in the midline at an angle of 30 degrees to the vertical. This resulted in placement of the tip of the needle just anterior to the optic chiasm. After 30 minutes of equilibration with adjustment of the level of anesthesia to obtain a mean arterial blood pressure in the range 80 to 100 mmHg, 250 µL of blood was withdrawn from the arterial catheter and injected manually into the prechiasmatic cistern at a pressure equal to the mean arterial blood pressure. The rat was kept under anesthesia for another 60 minutes to allow recovery from the cerebral insult, after which time the catheters were removed and the incisions closed. The rat was then extubated and allowed to recover. In addition, a series of sham-operated rats were prepared. They went through the same procedure except that no blood was injected. Thirty-five rats were operated upon. Two rats died prematurely after 1 day of observation.

Harvest of Cerebral Arteries

After 2 days of observation, the rats were sacrificed. Under microscopic vision, the middle cerebral, posterior communicating, and basilar arteries were carefully dissected free and cut into 1 mm long cylindrical segments. Care was taken to maintain an intact endothelial cell layer.

In Vitro Pharmacology

Methods for pharmacological studies in vitro have been described.[2] The segments were mounted on two metal wires 40 |xm in diameter (Myograph, J.P. Trading, Denmark) with one fixed and the other connected to a force displacement transducer. Experiments were run in an isotonic temperature controlled (37°C) buffer solution. Tension was recorded on a PowerLab unit (AD Instruments, Oxford, UK) using the program Chart. The vessels were stretched to an initial resting tone of 2 mN and then allowed to stabilize at this tone for 1 hour. The contraction induced by K+, 60 mmol/L, was used as a measure of tissue contractile capability and as reference for subsequent contractile experiments. Concentration–contraction curves were constructed with ET-1 in the concentration range 10^{-14} to 10^{-7} mol/L and sarafotoxin 6c (S6c) in the range 10^{-12} M to 10^{-7} mol/L. ET-1 was used as a general endothelin receptor agonist and S6c as a selective ET_B agonist.[4] To further characterize the contractile response, the effect of ET-1 was tested against the ET_A receptor selective antagonist FR139317 and the ET_B receptor selective antagonist IRL1038.

Real-Time RT-PCR Method

To quantify messenger ribonucleic acid (mRNA) for ET_A and ET_B receptors, real-time reverse transcriptase polymerase chain reaction (RT-PCR) was employed as previously described.[1] The arterial samples were pooled. Total RNA from the vessels was extracted using TRIzol® agent after mechanical homogenization. The reverse transcriptase synthesis of cDNA was done in a 20 µL RT reaction volume. Quantitative real-time PCR was performed in a GeneAmp 5700 Sequence Detection System (Applied Biosystems, Foster City, CA, USA) using GeneAmp SYBR Green with the cDNA synthesized above as template in a 50 . L reaction volume. A blank control was included in all experiments. Specific primers for the rat ET_A and ET_B receptors were designed using Primer Express (ver. 2.0) software (Applied Biosystems, USA). The RNA for the housing keeping gene elongation factor 1 was used as reference. Four separate runs were performed for each measurement.[1,5]

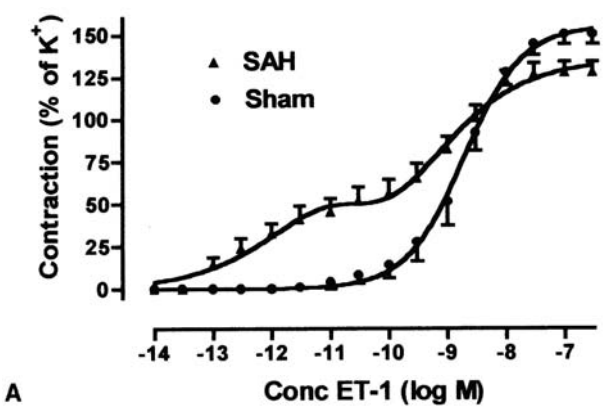
Calculation and Statistics

Data are expressed as mean ± standard error of the mean (SEM). Contractile responses in each segment are expressed as a percent of the contraction induced in that segment by K+, 60 mmol/L. In a given experiment E_{max} denotes the maximum contractile response elicited and pEC_{50} the negative logarithm of the concentration that elicits half the maximum response. For biphasic responses, $E_{max(1)}$ and $pEC_{50(1)}$ are used to describe the high affinity phase and $E_{max(2)}$ and $pEC_{50(2)}$ to describe the low affinity phase. The statistical significance of differences observed was calculated using Student's *t*-test using a probability level of .05 as the level of significance.

Results

In Vitro Pharmacology

In the middle cerebral artery from animals with SAH, ET-1 produced a significantly enhanced biphasic dose-dependent response with an initial $E_{max(1)}$ of 50 ± 8%, a $pEC_{50(1)}$ of 12.2 ± 0.3 and in higher concentrations of ET-1 an $E_{max(2)}$ of 127 ± 6% and a $pEC_{50(2)}$ of



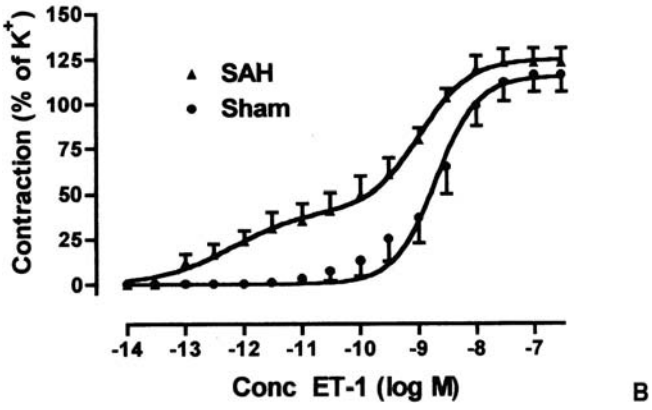


FIGURE 18–1 (A) Concentration–contraction curves showing the effect of endothelin (ET)-l on the middle cerebral artery and (B) the basilar artery, respectively, from rats subjected to subarachnoid hemorrhage (SAH) or to sham operation. In both arteries an enhanced ET-1 response is observed in rats with experimental SAH compared with sham operated rats (values are means ± SEM).

9.3 ± 0.1 (Fig. 18–1). In comparison, middle cerebral arteries from sham-operated rats gave rise to an E_{max} of 150 ± 7% and a pEC_{50} of 8.7 ± 0.3. Responses in the basilar artery were similar to those obtained in the middle cerebral artery. Thus, ET-1 produced a biphasic, dose-dependent, contractile response with an $E_{max(1)}$ of 41 ± 10%, a $pEC_{50(1)}$ 12.2 ± 0.2, an $E_{max(2)}$ 123 ± 8%, and a $pEC_{50(2)}$ of 9.0 ± 0.1. These values were significantly different from sham-operated rats, where a normal sigmoidally shaped curve was obtained with an E_{max} of 116 ± 9% and a pEC_{50} of 8.7 ± 0.1. In contrast to the observations from the middle cerebral and basilar arteries, no difference in the contractile response to ET-1 in the posterior communicating artery was observed between rats with SAH and sham-operated rats. In all cases the ET-1-induced contraction curve in the arteries from SAH rats was significantly shifted to the right in the presence of FR139317, indicating an ET_A receptor-mediated response. In addition, the upregulated response in the middle cerebral and basilar arteries was attenuated by pretreatment with IRL1038 as well with S6c, indicating a functional ET_B receptor. Interestingly, however, S6c did not produce contraction itself.

Real-Time RT-PCR

Quantitative RT-PCR showed that the numbers of copies of mRNA coding for the ET_B receptor in the middle cerebral and basilar arteries were increased compared with the same type of arteries from sham operated rats (Fig. 18–2). Specifically, 3.4 ± 0.1 times more mRNA copies for the ET_B receptor were found in the middle cerebral and 6.9 ±1.0 times more in the basilar artery. In comparison, no significant difference was found in the posterior communicating artery. In addition, no change in the number of copies of mRNA coding for the ET_A receptor was found.

Conclusion

The functional and molecular data clearly indicate that experimental SAH induces the expression of a functional ET_B receptor. The appearance of this receptor is linked to an increase in sensitivity of the cerebral artery toward ET-1. This means that the artery will contract at much lower concentrations of ET-1 compared with normal arteries. The antagonist data indicate a complex relationship between the already

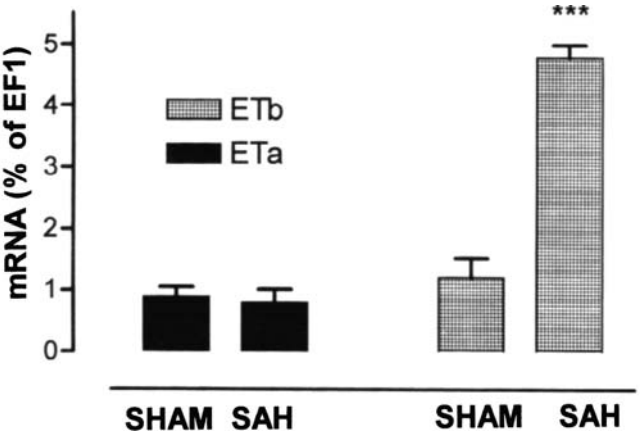


FIGURE 18–2 Bar graph showing the amount of endothelin A (ET_A) and ET_B receptor messenger ribonucleic acid (mRNA) compared with elongation factor-1 in arteries from rats with experimental subarachnoid hemorrhage (SAH) compared with sham-operated rats. The expression of the ET_B receptor is significantly (*** $p < .001$) augmented in arteries from rats with experimental SAH.

present ET_A receptor and the upregulated ET_B receptor. The results are novel, and although more data, especially involving human material, are needed, they indicate a possible end stage mechanism in cerebral vasospasm not hitherto appreciated.

REFERENCES

1. Stenman E, Malmsjö M, Uddman E, Gido G, Wieloch T, Edvinsson L. Cerebral ischemia upregulates vascular endothelin ET(B) receptors in rat. Stroke 2002;33:2311–2316
2. Hansen-Schwartz J, Edvinsson L. Increased sensitivity to ET-1 in rat cerebral arteries following organ culture. Neuroreport 2000;11:649–652
3. Prunell GF, Mathiesen T, Svendgaard N-A. A new experimental model in rats for study of the pathophysiology of subarachnoid hemorrhage. Neuroreport 2002;13:2553–2556
4. Hansen-Schwartz J, Edvinsson L. Endothelin. In: Edvinsson L, Krause D, eds. Cerebral Blood Flow and Metabolism. New York: Lippincott, Williams & Wilkins, 2002:466–479
5. Hansen-Schwartz J, Hoel NL, Zhou M, Xu C-B, Svendgaard N-A, Edvinsson L. Subarachnoid hemorrhage enhances endothelin receptor expression and function in rat cerebral arteries. Neurosurgery 2003;52:1188–1194

19

Endothelial Dysfunction in a Primate Model of Cerebral Vasospasm

BRIAN A. IULIANO, M.D., RYSZARD M. PLUTA, M.D., CARLA S. JUNG, M.D., EDWARD H. OLDFIELD, M.D.

Abstract

There is a relative absence of prior in vivo studies of endothelial function of cerebral vessels following subarachnoid hemorrhage (SAH). We thus decided to investigate endothelium-dependent responses in a primate model of vasospasm after SAH. Endothelial function was assessed in terms of vascular responses to intracarotid injection of various drugs known to act via the endothelium. Seventeen adult male cynomolgus monkeys were used. Cerebrovascular endothelium–dependent responses were examined in control animals (n = *7)* and in animals 7 (n = 6), 14 (n = 3), and 21 (n = 2) days after placement of a subarachnoid clot around the right middle cerebral artery. Cortical cerebral blood flow and cerebrovascular resistance were recorded continuously during 5-minute intracarotid infusions of 5% dextrose vehicle, acetylcholine, histamine, bradykinin, and calcimycin. In control animals intracarotid acetylcholine infusion produced a significant 7.8% increase in cerebral blood flow and a 9.3% reduction in cerebrovascular resistance in comparison with infusion of vehicle. These responses were lost in animals 7 days after SAH, specifically in the subset of animals with vasospasm. Calcimycin infusion resulted in a significant reduction in cerebral blood flow and an increase in cerebrovascular resistance in animals 7 days after SAH and in animals with vasospasm. Intracarotid acetylcholine produced a measurable physiological response in the normal primate cerebrovasculature. Cerebral vasospasm after SAH produced a pathophysiological effect similar to endothelial denudation that has been noted in experiments in vitro. The change in the vascular response to acetylcholine and calcimycin in animals with vasospasm compared with controls provides evidence that endothelial dysfunction plays a key role in the development and/or sustenance of vasospasm after SAH.

Endothelial injury, or dysfunction, is one potential factor involved in the pathophysiology of cerebral vasospasm. Abnormalities in endothelial control of vascular tone, or endothelial dysfunction, have been demonstrated in several disease processes including hypertension, atherosclerosis, diabetes mellitus, coronary artery disease, congestive heart failure, and cerebral vasospasm. Several studies have examined endothelial-dependent processes in animal models of subarachnoid hemorrhage (SAH) and have shown abnormalities in endothelial-dependent vascular relaxation. However, these studies have been performed in vitro or in nonprimate models.

In this experiment we examine the hypothesis that there is an abnormality in endothelial-dependent vascular relaxation that is associated with cerebral vasospasm after SAH. Our objective was to assess endothelial-dependent vascular relaxation in a primate model of cerebral vasospasm in vivo. We carefully selected drugs with a short half-life and rapid total body clearance so that delivery of the drugs via intracarotid infusions limited the drug effect to the cerebral vasculature and avoided the physiological changes produced by pharmacologically active systemic levels.

Materials and Methods

The animal study proposal was reviewed by the Animal Care and Use Committee of the National Institutes of Neurological Disorders and Stroke and met National Institutes of Health guidelines for animal care. This study was designed to investigate endothelium-mediated vasorelaxation in the territory of the middle cerebral artery in a primate model of cerebral vasospasm. Vascular responses were monitored by recording changes in cerebral blood flow, cerebral vascular resistance, and arteriographic middle cerebral artery size evoked by intracarotid drug infusions. Intracarotid infusions of acetylcholine (10^{-4} mol/L), histamine (10^{-5} mol/L), bradykinin (10^{-6} mol/L), and calcimycin (10^{-5} mol/L) were performed in control animals (n = 7) and in primates 7 (n = 6), 14 (n = 3), and 21 (n = 1) days after placement of an arterial blood clot around the surgically exposed right middle cerebral artery. The control group included naive and sham-operated animals. The animals 14 and 21 days after SAH were combined into one group (SAH 14/21). Drugs were prepared in 5% dextrose solution and infused at a rate of 1.3 mL/min to achieve an intracarotid concentration one tenth of the concentration in the infused solution. Cerebral blood flow was recorded continuously with a laser Doppler cerebral blood flow probe. Cerebral arteriography was performed immediately after completing each drug infusion. Several recordings were performed on each animal with at least 15 minutes between infusions. All experiments were performed under controlled physiological conditions.

For each drug infusion, cerebral blood flow and mean arterial pressure were averaged for a 10-second preinfusion baseline period and for each minute during the infusion. Cerebral vascular resistance was calculated as the mean systemic arterial blood pressure divided by cerebral blood flow. The final results are reported as the percent change from baseline. For statistical analysis, values were compared by analysis of variance (ANOVA) with Fisher's protected least squares difference (PLSD). Significance was accepted at a probability value < .05.

Results

The mean degree of vasospasm for the group 7 days after SAH was 36% (range, 2–75%). Significant vasospasm (> 25%) was present in four of six animals in this group. One week after blood clot placement, three of four animals in the day 14/21 group had significant vasospasm, which resolved in all animals by the day of the final study. No control animals had significant middle cerebral artery vasospasm at the time of recording of vascular responses.

Changes in blood flow and cerebrovascular resistance after 5-minute intracarotid infusions of dextrose, acetylcholine, histamine, bradykinin, and calcimycin were first assessed in control animals. Dextrose infusion produced a 4.6% reduction in cerebral blood flow with a corresponding 3.6% increase in cerebrovascular resistance. In comparison with dextrose infusion, acetylcholine infusions produced a 7.8% increase in cerebral blood flow and a 9.3% reduction in cerebrovascular resistance (Fig. 19–1). Histamine infusion produced a 7.8% increase in cerebral blood flow (p < .01) but also produced a 5.8% increase in systemic mean arterial pressure (p < .001). Histamine infusion did not produce a significant change in cerebrovascular resistance. Bradykinin and calcimycin infusions produced no statistically significant changes in cerebral blood flow, mean arterial pressure, or cerebrovascular resistance. Dextrose, acetylcholine, histamine, bradykinin, and calcimycin infusions produced no

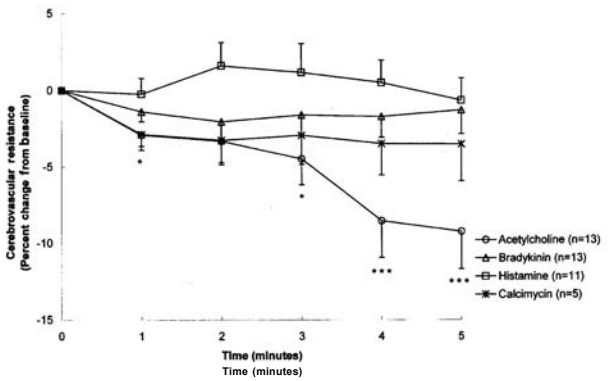


FIGURE 19–1 Graph depicting changes in cerebrovascular resistance during 5-minute intracarotid infusions of experimental drugs in control animals. Points represent a mean value of several intracarotid infusions for each drug plotted at 1-minute intervals minus the corresponding value for 5% dextrose vehicle infusion. Each point represents the percent change in cerebrovascular resistance compared with the value at the start of that infusion. Error bars represent standard error (*p < .05, ***p < .001).

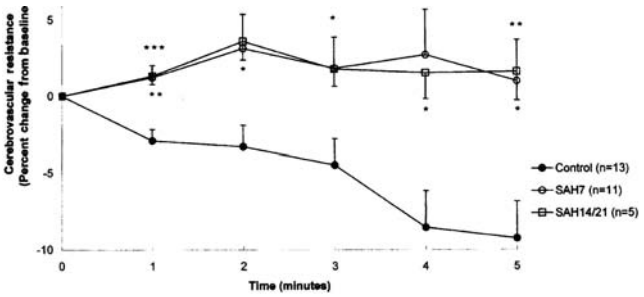


FIGURE 19–2 Graph depicting changes in cerebrovascular resistance during 5-minute intracarotid infusions of acetylcholine in control animals and animals 7 or 14/21 days after subarachnoid hemorrhage (SAH). Points represent the mean value of several intracarotid infusions plotted at 1-minute intervals minus the corresponding value for 5% dextrose vehicle infusion. Each point represents the percent change compared with the value at the start of the infusion. Error bars represent standard error (*$p < .05$, **$p < .01$, ***$p < .001$).

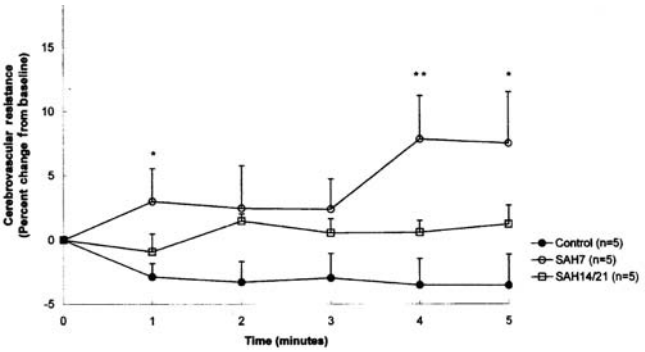


FIGURE 19–3 Graph depicting changes in cerebrovascular resistance during 5-minute intracarotid infusion of calcimycin in control animals and animals 7 or 14/21 days after subarachnoid hemorrhage (SAH). Points represent the mean value of several intracarotid infusions plotted at 1-minute intervals minus the corresponding value for 5% dextrose vehicle infusion. Each point represents the percent change compared with the value at the start of the infusion. Error bars represent standard error (*; $p < 0.05$, **; $p < 0.01$).

significant change in the arteriographic middle cerebral artery diameter measurements.

The cerebral blood flow and cerebrovascular resistance responses to dextrose infusion were unchanged in animals 7, 14, and 21 days after SAH compared with controls.

The cerebral blood flow increase seen in response to acetylcholine in control animals was completely lost in animals 7 days after SAH ($p < .01$) and specifically in animals with vasospasm ($p < .01$) compared with control animals. The corresponding decrease in cerebrovascular resistance also was completely lost in animals 7 days after SAH ($p < .006$), and in those with vasospasm ($p < .01$, Fig. 19–2).

Histamine and bradykinin infusions produced no statistically significant changes 7, 14, or 21 days after SAH. Neither drug produced significant changes in cerebral blood flow or cerebrovascular resistance in animals with or without vasospasm on day 7. In animals 7 days after SAH (Fig. 19–3) and in animals with vasospasm on day 7 after SAH, calcimycin infusion produced a significant increase, 11.2% ($p < .04$) and 13.9% ($p < .04$), respectively, in cerebrovascular resistance that did not occur in control animals, in animals 14/21 days after SAH, or in animals without vasospasm on day 7 after SAH. This change in the cerebrovascular resistance was associated with a corresponding decrease in the cerebral blood flow in comparison with the control group. There was no significant change in arteriographic middle cerebral artery area in response to the dextrose, acetylcholine, histamine, bradykinin, and calcimycin infusions after SAH.

Conclusion

Chronic cerebral vasospasm following aneurysmal SAH is a result of an aberration in the control and maintenance of normal vascular tone and diameter, which, depending on its severity and duration, results in reduced cerebral blood flow and ischemic neurological deficits. Our results demonstrate an alteration in endothelium-dependent vasomotor responses to acetylcholine and calcimycin in primates with angiographically documented vasospasm 7 days after SAH. Endothelial dysfunction is present in vivo in a primate model of cerebral vasospasm, and this loss of normal endothelial function may play a key role in the development of chronic vasospasm after SAH.

20

Increased Contractile Effect of Endothelin-1 on Isolated Rat Basilar Artery after Experimental Subarachnoid Hemorrhage

HARTMUT VATTER, M.D., EDGAR DETTMANN, M.D., SUMBELE NGONE, M.D., BETTINA LANGE, M.D., ANDREAS RAABE, M.D., VOLKER SEIFERT, M.D., PH.D., MICHAEL ZIMMERMANN, M.D., PH.D.

Abstract

Increased synthesis of endothelin-1 (ET-1) after subarachnoid hemorrhage (SAH) is considered one of the major pathophysiological factors for the development of cerebral vasospasm. Whether the vasomotor effects of ET-1 on the cerebrovasculature are additionally altered during vasospasm still remains unclear. Therefore, in the present study the contractile effect of ET-1 and big ET-1 was characterized on cerebral vessels after experimental SAH. Male Sprague-Dawley rats received 0.2 mL arterial blood in the cisterna magna twice, repeated to induce vasospasm. Animals were sacrificed on 7 days after SAH, and the basilar artery was dissected meticulously. Ring segments from the basilar artery were prepared for the measurement of isometric force in an organ bath. Concentration-effect curves were constructed for basilar artery ring segments from rats after SAH and controls by cumulative application of ET-1 (10^{-12} –3×10^{-7} mol/L) or big ET-1 (10^{-10} – 10^{-6} mol/L). Both ET-1 and big ET-1 induced a dose-dependent contraction of vessels with and without vasospasm. The pD_2 values for ET-1 were 7.43 ± 0.12 (SAH) and 7.36 ± 0.02 (control) and did not differ significantly. In contrast, E_{max} after SAH significantly increased to 126 ± 8% (control: 105 ± 12%). Similarly, E_{max} for big ET-1 was enhanced significantly after SAH. Values were 125 ± 12% (SAH) and 97 ± 14% (control). The pD_2 value for big ET-1, however, was significantly reduced in vasospastic arteries [6.71 ± 0.06 (SAH) vs 7.06 ± 0.17 (control)]. The present data indicate an enhanced contractile effect of ET-1 and big ET-1 on cerebral vessels with vasospasm after SAH, which supports a role for ET-1 in the development of vasospasm. Furthermore, a decrease of functional ET-converting enzyme activity of cerebral vessels after SAH may be suggested.

Endothelin-1 (ET-1) is considered to be a key pathophysiological factor responsible for the development of delayed cerebral vasospasm after subarachnoid hemorrhage (SAH). A correlation between the development of vasospasm in patients suffering from spontaneous SAH due to the rupture of an aneurysm and an increase in the levels of ET-1 and its precursor big ET-1 in the cerebrospinal fluid was reported previously.[1–3] Furthermore, ET-1 induces a long-lasting and potent contraction of cerebral blood vessels.[4–6] Increased

concentrations of ET-1 or big ET-1 detected in the cerebrospinal fluid, however, were of insufficient levels to produce contraction of cerebral arteries in vitro.[1–3] ET-1 acts on cerebral vessels by two specific receptors that are termed ET_A and ET_B receptors.[7] The contractile effect of ET-1 is mediated by the ET_A receptor that is located on smooth muscle cells,[4] whereas activation of the ET_{B1} receptor on endothelial cells results in nitric oxide–dependent vasodilatation.[8] There are data, however, to suggest the existence of a contractile ET_{B2} receptor in the cerebrovasculature after SAH, which results in altered vasomotor effects of ET-1 during vasospasm.[9,10]

It also is of note that, in addition to ET receptor antagonists, the ET-converting enzyme represents a promising target for treatment of ET-dependent vasoconstriction. There are reports that experimental vasospasm was successfully treated with ET-receptor antagonists[11,12] and inhibitors of the ET-converting enzyme.[13,14] Therefore, the aim of the present study was to characterize the functional ET-converting enzyme activity and the vasomotor effects of ET-1 on isolated cerebral arteries in an established model of delayed cerebral vasospasm.[15]

Materials and Methods

Vasospasm was induced by a modified double hemorrhage model in male Sprague-Dawley rats as established by Solomon et al.[15] The animals received two injections of 0.2 mL autologous arterial blood into the cisterna magna on days 1 and 2. Anesthesia during the procedure was induced and maintained with intraperitoneal application of midazolam and ketamine. During the observation period the animals received buprenorphine (0.2 mg/kg) daily for analgesia. The amount and the distribution of the subarachnoid blood was evaluated by T2- and T2*-weighted magnetic resonance imaging (Phillips Gyroscan, 1.5 Tesla using a Synergy flex M coil, Phillips Medical Systems, Andover, MA) 4 days after the first SAH. Vasospasm was documented by selective vertebrobasilar angiography 7 days after SAH. Animals without a sufficient distribution of subarachnoid blood or without significant vasospasm were excluded from further investigations. After documentation of the presence of vasospasm, animals were sacrificed, and the basilar artery was dissected meticulously. Ring segments from the basilar artery were prepared for measurement of isometric force in an organ bath. The functional integrity of the musculature and the endothelium was tested as described previously.[8] Concentration-effect curves were constructed for ET-1 and big ET-1 in animals with vasospasm and controls by cumulative application of the respective compound. Contractions were expressed as a percent of a reference contraction induced by Krebs solution containing K+, 124 mmol/L. For comparison of the concentration-effect curves, the E_{max} and pD_2 ($-\log EC_{50}$) values were used, and shifts of the EC_{50} value were calculated. Statistical analysis was performed using Student's *t*-test for comparison of mean values.

Results

ET-1 induced a dose-dependent contraction in ring segments from animals after SAH and in controls (Fig. 20–1). The onset and the position of the concentration effect curve was not significantly altered by vasospasm after SAH. The pD_2 values were 7.43 ± 0.12 (SAH group) and 7.36 ± 0.03 (control) and did not differ significantly. Maximum contraction, in contrast, was significantly enhanced after SAH. The E_{max} values were 126 ± 8% (SAH) and 105 ± 12% (control), respectively. Similar to ET-1, its precursor big ET-1 induced a dose-dependent contraction in vasospastic and control segments (Fig. 20–2). The concentration-effect curve for big ET-1 was shifted to the right by a factor of 2.2 in animals with vasospasm. Accordingly, the pD_2 value was significantly reduced after experimental SAH (6.71 ± 0.06) compared with the control

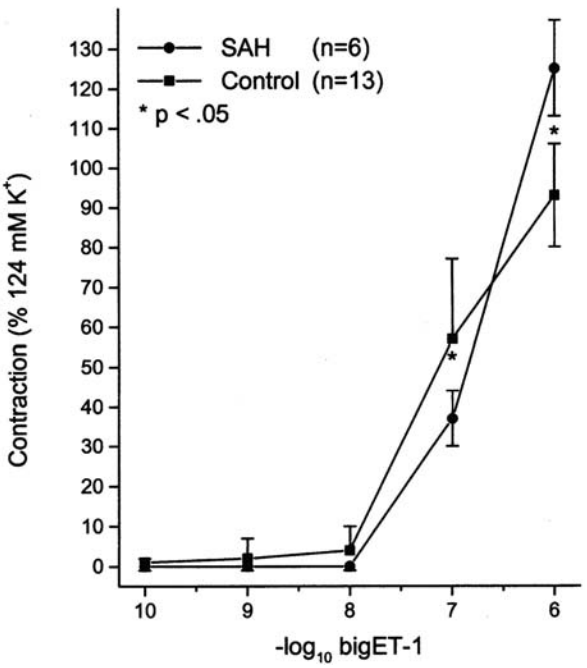


FIGURE 20–1 Concentration effect curves for endothelin (ET)-l obtained in ring segments from animals suffering from subarachnoid hemorrhage (SAH) and from controls. Values are means ± standard deviations. The concentration-effect curve was not shifted in the animals with cerebral vasospasm (SAH group). In contrast, the maximum contraction was significantly enhanced after experimental SAH (* $p < .05$).

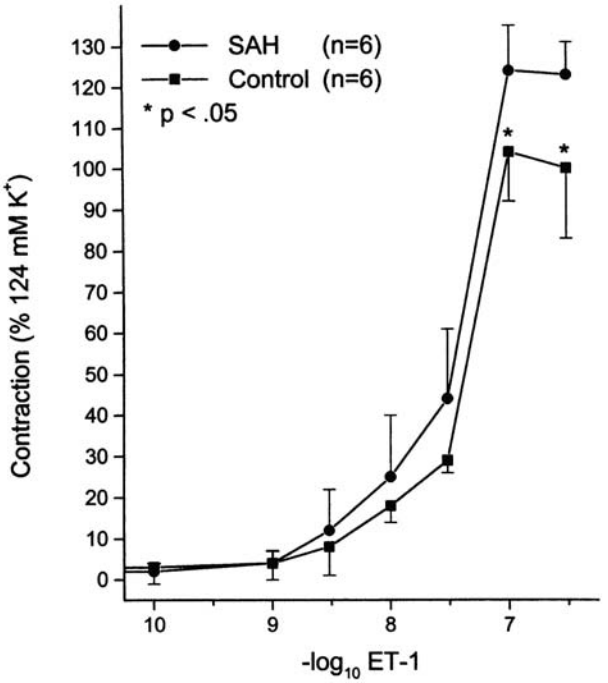


FIGURE 20–2 Concentration-effect curves for big endothelin (ET)-l obtained in ring segments from animals suffering from cerebral vasospasm after subarachnoid hemorrhage (SAH) and from controls. The concentration-effect curve for big ET-1 was shifted to the right in animals with vasospasm after SAH. Similar to ET-1, the maximum contraction was significantly enhanced after SAH (* $p < .05$, values are means ± standard deviations).

value (7.06 ± 0.17). The E_{max} values were 125 ± 12% (SAH) and 97 ± 14% (control) and differed significantly. This enhanced contraction was similar to the corresponding increase seen with ET-1. The shift of the concentration-effect curve for ET-1 versus big ET-1 was 2.0 in control segments and 5.2 in segments from animals after SAH.

Discussion and Conclusion

The present experiments in a rat model of SAH show that the concentration-effect curve and the onset of contraction in response to ET-1 was not significanty shifted by SAH and vasospasm. In contrast, the maximum contraction was enhanced after experimental SAH. Therefore, the present data support the hypothesis that after SAH there is either or both an upregulation of contractile ET_A receptors and some type of alteration in the second messenger cascade that is activated in response to ET-1 binding to these receptors. This is likely more important than the. presence of contractile ET_{B2} receptors. The discrepancy between these and previous observations[10] may be explained on the basis that the arteries examined in this study were obtained 7 days after SAH and wei roven to have delayed vasospasm. Prior studies examined arteries only a short time after SAH and did not determine whether the arteries actually had vasospasm.[10]

Big ET-1 itself does not bind to any of the known ET receptors.[16] Therefore, its biological activity requires the presence of functional ET-converting enzyme activity. This activity can be estimated in a semiquantitative fashion by the shift in concentration-effect curves between ET-1 and big ET-1.[16,17] The increase of this shift from 2.0 in the control rats to 5.2 after experimental SAH in the present study may, therefore, suggest an alteration of functional ET-converting enzyme activity in cerebral vessels that have vasospasm after SAH. Further investigations, such as a biochemical analysis of actual enzyme activity, are necessary to prove this hypothesis. In addition, while there is some evidence that ET-converting enzyme inhibitors may reduce experimental vasospasm, more specific drugs need to be tested.[13] The present results may, therefore, be taken to support the use of selective ET_A receptor antagonists as the most promising approach for the treatment of vasospasm.

REFERENCES

1. Seifert V, Loffler BM, Zimmermann M, Roux S, Stolke D. Endothelin concentrations in patients with aneurysmal subarachnoid hemorrhage: correlation with cerebral vasospasm, delayed ischemic neurological deficits, and volume of hematoma. J Neurosurg 1995;82:55–62
2. Suzuki R, Masaoka H, Hirata Y, Marumo F, Isotani E, Hirakawa K. The role of endothelin-1 in the origin of cerebral vasospasm in patients with aneurysmal subarachnoid hemorrhage. J Neurosurg 1992;77:96–100
3. Ehrenreich H, Lange M, Near KA, et al. Long-term monitoring of immunoreactive endothelin-1 and endothelin-3 in ventricular cerebrospinal fluid, plasma, and 24-h urine of patients with subarachnoid hemorrhage. Res Exp Med (Berl) 1992;192:257–268
4. Feger GI, Schilling L, Ehrenreich H, Wahl M. Endothelin-induced contraction and relaxation of rat isolated basilar artery: effect of BQ-123. J Cereb Blood Flow Metab 1994;14:845–852
5. Papadopoulos SM, Gilbert LL, Webb RC, D'Amato CJ. Characterization of contractile responses to endothelin in human cerebral arteries: implications for cerebral vasospasm. Neurosurgery 1990;26:810–815
6. Vatter H, Mursch K, Zimmermann M, et al. Endothelin-converting enzyme activity in human cerebral circulation. Neurosurgery 2002;51:445–452
7. Rubanyi GM, Polokoff MA. Endothelins: molecular biology, biochemistry, pharmacology, physiology, and pathophysiology. Pharmacol Rev 1994;46:325–415
8. Schilling L, Feger GI, Ehrenreich H, Wahl M. Endothelin-3-induced relaxation of isolated rat basilar artery is mediated by an endothelial ETB-type endothelin receptor. J Cereb Blood Flow Metab 1995;15:699–705
9. Zuccarello M, Boccaletti R, Romano A, Rapoport RM. Endothelin B receptor antagonists attenuate subarachnoid hemorrhage-induced cerebral vasospasm. Stroke 1998;29:1924–1929
10. Hansen-Schwartz J, Hoel NL, Zhou M, Xu CB, Svendgaard NA, Edvinsson L. Subarachnoid hemorrhage enhances endothelin receptor expression and function in rat cerebral arteries. Neurosurgery 2003;52:1188–1195

11. Zimmermann M, Seifert V, Loffler BM, Stolke D, Stenzel W. Prevention of cerebral vasospasm after experimental subarachnoid hemorrhage by RO 47–0203, a newly developed orally active endothelin receptor antagonist. Neurosurgery 1996;38:115-120
12. Wanebo JE, Arthur AS, Louis HG, et al. Systemic administration of the endothelin: a receptor antagonist TBC 11251 attenuates cerebral vasospasm after experimental subarachnoid hemorrhage: dose study and review of endothelin-based therapies in the literature on cerebral vasospasm. Neurosurgery 1998;43:1409-1418
13. Caner HH, Kwan AL, Arthur A, et al. Systemic administration of an inhibitor of endothelin-converting enzyme for attenuation of cerebral vasospasm following experimental subarachnoid hemorrhage. J Neurosurg 1996;85:917-922
14. Zimmermann M, Jung C, Raabe A, Spanehl O, Fach K, Seifert V. Inhibition of endothelin-converting enzyme activity in the rabbit basilar artery. Neurosurgery 2001;48:902–910
15. Solomon RA, Antunes JL, Chen RY, Bland L, Chien S. Decrease in cerebral blood flow in rats after experimental subarachnoid hemorrhage: a new animal model. Stroke 1985;16:58–64
16. Parnot C, Le Moullec JM, Cousin MA, Guedin D, Corvol P, Pinet F. A live-cell assay for studying extracellular and intracellular endothelin-converting enzyme activity. Hypertension 1997;30:837–844
17. Schilling L, Vatter H, Mursch K, Ehrenreich H, Schmiedek P. Characterization of the contractile and relaxant action of the endothelin-1 precursor, big endothelin-1, in the isolated rat basilar artery. Peptides 2000;21:91–99

21

CSF Levels of ADMA, an Endogenous Inhibitor of Nitric Oxide Synthase, Are Associated with Vasospasm After Subarachnoid Hemorrhage

CARLA S. JUNG, M.D., BRIAN A. IULIANO, M.D., JUDITH HARVEY-WHITE, B.S., EDWARD H. OLDFIELD, M.D., RYSZARD M. PLUTA, M.D., PH.D.

Abstract

Endothelial dysfunction has been observed after subarachnoid hemorrhage (SAH). In addition, recent studies have documented the existence of a symmetric dimethyl-L-arginine (ADMA), an endogenous inhibitor of endothelial nitric oxide synthase. We sought to determine if ADMA is detectable after SAH and if alterations in ADMA levels are associated with cerebral vasospasm in a primate model of SAH and vasospasm. Levels of ADMA were determined by high performance liquid chromatography in cerebrospinal fluid (CSF) and serum collected from monkeys at baseline and on days 7,14, or 21 after SAH. Cerebral arteriograms were performed to assess the degree of vasospasm. In monkeys, CSF ADMA levels remained unchanged in the control group ($n = 6$) and in animals without vasospasm after SAH ($n = 3$; $p = .57$). CSF ADMA was significantly increased in animals with vasospasm ($n = 10$). ADMA increased on day 7 after SAH ($p < .02$) and decreased on days 14 through 21 ($p < .05$). CSF ADMA levels were correlated with the degree of vasospasm (correlation coefficient = 0.8; $p < .0001$). Serum ADMA levels remained unchanged and were not associated with the degree of vasospasm. In a primate model of SAH, the levels of ADMA in CSF significantly increased concurrent with the development of vasospasm. These results suggest that endogenous inhibition of endothelial nitric oxide synthase by ADMA may be involved in the development of cerebral vasospasm after SAH.

Delayed cerebral vasospasm leading to ischemic neurological deficit is a major cause of morbidity and mortality in patients with subarachnoid hemorrhage (SAH).[1,2] The pathogenesis of vasospasm remains unclear despite extensive experimental and clinical research. Nitric oxide (NO) is a powerful vasodilator that plays a major role in the regulation of cerebral vascular tone.[3–5] Recent studies indicate that SAH leads to decreased availability of NO, reducing endothelium-dependent and NO-mediated relaxation of large conductive cerebral arteries.[467] Following SAH, decreased levels of cyclic guanosine monophosphate were described in the arteries that were vasospastic.[6,8] However, only decreased neuronal nitric oxide synthase (nNOS) immunoreactivity has been reported after SAH, whereas immunoreactivity for endothelial

NOS (eNOS) and levels of eNOS messenger ribonucleic acid remain unchanged or are increased.[4,8] Together these findings suggest that eNOS activity may be inhibited in association with development of delayed vasospasm.

Asymmetric dimethyl L-arginine (ADMA) has been identified as an endogenous inhibitor of eNOS.[9,10] ADMA levels are elevated in various disease states in which a reduction of NO production contributes to pathology, including hypertension, renal failure, and hypercholesterolemia.[11-12] We hypothesized that the decreased availability of NO in vasospasm after SAH may be evoked by inhibition of eNOS by ADMA. To examine this hypothesis, levels of ADMA were measured in cerebrospinal fluid (CSF) from a primate model of delayed cerebral vasospasm.

Material and Methods

The animal protocol was reviewed by the Institutional Animal Care and Use Committee and met the National Institutes of Health guidelines for animal care. Nineteen cynomolgus monkeys were divided into two groups to serve as controls (n = 6) or to undergo creation of SAH (n = 13). The animals were placed under general anesthesia and then underwent a right frontotemporal craniectomy under aseptic conditions. The proximal 14 mm of the right middle cerebral artery was exposed, and 5 mL of preclotted, autologous arterial blood was placed around the artery. On days 0 (day of SAH), 7,14, and 21 cerebral arteriograms were performed to assess the degree of vasospasm. The area of the proximal 14 mm of the right middle cerebral artery was measured on the anteroposterior view of the arteriograms by three blinded examiners using a computerized image analysis system, as described elsewhere.[13] Arteriographic vasospasm was quantified relative to each animal baseline arteriogram and was classified as no vasospasm (< 11% reduction in area of the right middle cerebral artery), mild (11–25%), moderate (26–50%), and severe (> 50%). CSF was collected on days 0, 7, 14, and 21 after SAH, and levels of free ADMA were determined by high-performance liquid chromatography.

Results

The control animals (three naive and three sham) and three animals with SAH did not develop vasospasm. The remaining animals with SAH (n = 10) developed vasospasm. The degree of vasospasm peaked on day 7 (p < .001; two severe, three moderate, two mild vasospasm), significantly decreased on day 14 (p < .02; two moderate, three mild) and resolved by day 21 (p < .01; three no vasospasm, Fig. 21–1). Free ADMA

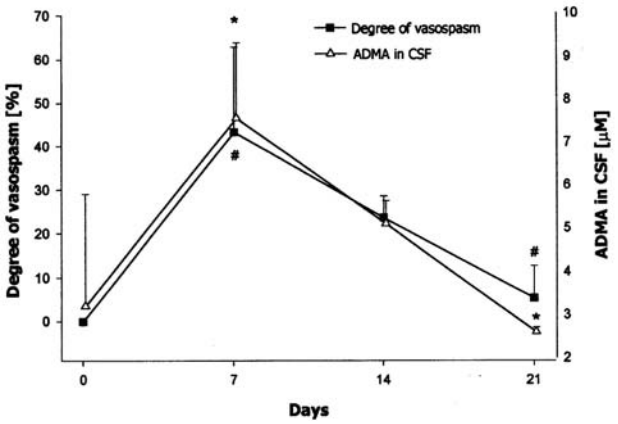


FIGURE 21–1 Graph showing the degree of vasospasm in animals that developed vasospasm after subarachnoid hemorrhage (SAH) (expressed as percent of the area of the proximal 14 mm of the right middle cerebral artery on the day after SAH compared with baseline on day 0) and the concentration of asymmetric dimethyl-L-arginine (ADMA) in the cerebrospinal fluid (CSF) [in µmol/L (. M)], by day after SAH. Values are means ± standard deviations. There was a significant difference in the degree of vasospasm between day 7 after SAH and day 0 and between day 7 and day 21 (#, p < .01). There was significant increase in ADMA levels in animals with vasospasm on day 7 after SAH and significant decrease from day 7 to days 14 and 21 (* p < .05).

levels were detectable in the CSF. ADMA levels were similar in the control and SAH animals on day 0 after SAH. On day 7, ADMA levels increased significantly in the SAH group (p < .05) and then decreased on days 14 and 21. The increase in ADMA on day 7, however, occurred only in animals with vasospasm (see Fig. 21–1). ADMA levels in monkeys with vasospasm increased from day 0 to day 7 (p < .02) and decreased on days 14 and 21 (p < .04). ADMA levels in animals without vasospasm remained unchanged after SAH (p = .57) and were significantly lower compared with ADMA levels in animals with vasospasm on day 7 (p < .03). ADMA levels in the CSF were strongly correlated with the degree of vasospasm (correlation coefficient = 0.7, p = .0002).

Conclusion

In a primate model of delayed vasospasm after SAH, the levels of ADMA in the CSF increased significantly in monkeys with vasospasm on day 7 and decreased on days 14 and 21. The changes in CSF ADMA followed the same time course as that of cerebral vasospasm in monkeys[14] and humans.[15] Furthermore, the degree of arteriographic vasospasm and the concentration of ADMA in the CSF were highly correlated. Moreover, the levels of ADMA in the CSF of monkeys with vasospasm are similar to concentrations that are

sufficient to constrict arteries in vivo. In rats and rabbits exogenously administered ADMA in concentrations of 10 to 300 μmol/L produces vasoconstriction.[16] Furthermore, experiments in vitro have shown that ADMA markedly inhibits vasodilation of animal and human vessel segments in response to acetylcholine without inhibiting vasodilation in response to sodium nitroprusside.[16,17] Although we have shown an association between vasospasm and CSF concentrations of ADMA, the relationship cannot be regarded as causal at this point. The results are, however, consistent with the hypothesis that endogenous inhibition of eNOS by ADMA may initiate or sustain delayed cerebral vasospasm after SAH. The involvement of ADMA in the etiology of SAH may open new approaches for treatment of cerebral vasospasm.

REFERENCES

1. Kassell NF, Torner JC, Haley EC Jr, et al. The International Cooperative Study on the Timing of Aneurysm Surgery, I: Overall management results. J Neurosurg 1990;73:18–36
2. Schievink WI. Intracranial aneurysms. N Engl J Med 1997;336:28–40
3. Iadecola C. Regulation of the cerebral microcirculation during neural activity: is nitric oxide the missing link? Trends Neurosci 1993;16:206–214
4. Pluta RM, Thompson BG, Afshar JK, et al. Nitric oxide and vasospasm. Acta Neurochir Suppl 2001;77:67–72
5. Thompson BG, Pluta RM, Girton ME, et al. Nitric oxide mediation of chemoregulation but not autoregulation of cerebral blood flow in primates. J Neurosurg 1996;84:71–78
6. Kim P, Schini VB, Sundt TM Jr, et al. Reduced production of cGMP underlies the loss of endothelium-dependent relaxations in the canine basilar artery after subarachnoid hemorrhage. Circ Res 1992;70:248–256
7. Suzuki Y, Kajita Y, Oyama H, et al. Dysfunction of nitric oxide in the spastic basilar arteries after subarachnoid hemorrhage. J Auton Nerv Syst 1994;49(suppl):S83–S87
8. Sobey CG, Faraci FM. Subarachnoid haemorrhage: what happens to the cerebral arteries? Clin Exp Pharmacol Physiol 1998;25:867–876
9. Kimoto M, Whitley GS, Tsuji H, et al. Detection of NG,NG-dimethylarginine dimethylaminohydrolase in human tissues using a monoclonal antibody. J Biochem (Tokyo) 1995;117:237–238
10. Leiper JM, Santa Maria J, Chubb A, et al. Identification of two human dimethylarginine dimethylaminohydrolases with distinct tissue distributions and homology with microbial arginine deiminases. Biochem J 1999;343:209–214
11. Boger RH, Sydow K, Borlak J, et al. LDL cholesterol upregulates synthesis of asymmetrical dimethylarginine in human endothelial cells: involvement of S-adenosylmethionine-dependent methyltransferases. Circ Res 2000;87:99–105
12. Vallance P, Chan N. Endothelial function and nitric oxide: clinical relevance. Heart 2001;85:342–350
13. Afshar JK, Pluta RM, Boock RJ, et al. Effect of intracarotid nitric oxide on primate cerebral vasospasm after subarachnoid hemorrhage. J Neurosurg 1995;83:118–122
14. Espinosa F, Weir B, Overton T, et al. A randomized placebo-controlled double-blind trial of nimodipine after SAH in monkeys, I: Clinical and radiological findings. J Neurosurg 1984;60:1167–1175
15. Weir B, Grace M, Hansen J, et al. Time course of vasospasm in man. J Neurosurg 1978;48:173–178
16. Faraci FM, Brian JE Jr, Heistad DD. Response of cerebral blood vessels to an endogenous inhibitor of nitric oxide synthase. Am J Physiol 1995;269:H1522–H1527
17. Segarra G, Medina P, Ballester RM, et al. Effects of some guanidino compounds on human cerebral arteries. Stroke 1999;30:2206–2210

22

Monitoring Activation of the Cerebral Endothelin System After Subarachnoid Hemorrhage by Measuring C-Terminal Fragment in Cerebrospinal Fluid

HARTMUT VATTER, M.D., MICHAEL ZIMMERMANN, M.D., PH.D., VOLKER SEIFERT, M.D., PH.D., LOTHAR SCHILLING, PH.D.

Abstract

Enhanced synthesis of endothelin-1 (ET-1) is considered to be an important factor in the development of cerebral vasospasm after subarachnoid hemorrhage (SAH). In the present investigation we measured the concentrations of ET-1 and its vasoinactive companion peptide, C-terminal fragment (CTF) in patients with SAH. CTF does not bind to ET_A receptors that mediate contractile functions or to the ET_B receptor that is presumed to be involved in clearance of ET. Samples of cerebrospinal fluid and plasma were obtained from five patients suffering from SAH (Hunt and Hess grade 4). Occurrence of delayed vasospasm was proven angiographically. The concentrations of ET-1 and CTF were analyzed by radioimmunoassay of samples obtained up to 7 days after bleeding. The concentration of CTF in plasma was higher than that of ET-1 by a factor of 3.4 ± 0.8 (mean ± SEM). There was no correlation between plasma CTF and development of vasospasm. In contrast, the concentration of CTF in cerebrospinal fluid was on average 47.5-fold higher than ET-1 ($p < .01$) and regularly exceeded the concentration in corresponding plasma samples. Average maximum values of CTF in cerebrospinal fluid were $8.3 \pm 0.4 \times 10^{-11}$ mol/L compared with 3.6 ± 1.4 X 10^{-11} mol/L for ET-1 ($p < .01$ vs CTF). Individually measured values of CTF amounted up to 2.4×10^{-10} mol/L in cerebrospinal fluid. Increases in CTF concentration preceded development of vasospasm. Given the equimolar generation of ET-1 and CTF, measuring CTF concentration in cerebrospinal fluid may give a superior indication of the activation of the cerebral ET system after SAH than ET-1. Maximum cerebrospinal fluid concentrations of CTF were in the range in which ET-1 exerts vasoconstriction. Thus, the present study suggests that the CTF concentration in cerebrospinal fluid may be a possible prognostic factor in the development of vasospasm.

Enhanced synthesis of endothelin-1 (ET-1) after subarachnoid hemorrhage (SAH) is considered to be a substantial pathophysiological factor for the development of delayed cerebral vasospasm. Data supporting this consideration are the potent and long-lasting contractile effect of ET-1 on cerebral vessels[1,2] and the successful reduction of experimental vasospasm by ET-receptor antagonists[3,4] or inhibitors of endothelin-converting enzyme.[5,6] Furthermore, a correlation between the vasospasm in patients suffering from SAH

and increasing levels of ET-1 or its precursor, big ET-1, in the cerebrospinal fluid (CSF) has been suggested previously.[7–9] Other investigations, however, could not confirm this correlation,[10,11] and these authors theorized that the elevations of ET-1 may reflect either or both damage and infarction of brain tissue after SAH.12

Synthesis of ET-1 begins with a pre-propeptide that is converted to the biologically inactive precursor, big ET-1.[13] This peptide is cleaved enzymatically by ET-converting enzymes to give ET-1, which exclusively mediates biological activity by binding to specific ET receptors. The other product of the peptide cleavage is C-terminal fragment (CTF).[14] Therefore, generation of ET-1 and its companion peptide CTF occurs in equimolar fashion. Whereas clearance of ET-1 appears to be at least in part due to internalization of the receptor–ligand complex after binding,[15] the fate of the biologically inactive CTF, which does not bind to any known receptor, remains uncertain in the CSF.

Therefore, the present study investigated whether the concentration of the CTF in plasma and CSF from patients suffering from SAH reflects the activity of the cerebral ET system in a better way than does ET-1 itself. A second goal was to examine whether CTF could be used as an indicator or even as a predictor for the development of vasospasm in these patients.

Materials and Methods

Samples of plasma and ventricular cerebrospinal fluid were obtained from five patients suffering from aneurysmal SAH. Patients were Hunt and Hess grade 4 and Fisher grade 3. In all cases the aneurysm responsible for the SAH was eliminated operatively after bleeding, and the patients received neurointensive care using a standard treatment protocol for SAH. All patients developed delayed cerebral vasospasm, and three patients developed cerebral infarction in spite of maximal conservative therapy. Patient 2 suffered an intraventricular hemorrhage on the sixteenth day after SAH, which was not related to the completely clipped aneurysm and the cause of which remains unclear. Further clinical details are given in Table 22–1.

Samples were obtained for up to 7 days after SAH. Concentrations of ET-1 and CTF were analyzed by radioimmunoassay. Values are means ± standard deviation.

Results

The concentration of CTF in plasma was higher than that of ET-1, on average by a factor of 3.4 ± 0.8. The time course of CTF concentration in plasma showed that there was a small increase between the second and fourth days after bleeding (Fig. 22–1). There was no apparent correlation with the development of vasospasm.

The time course of changes in concentrations of ET-1 and CTF in CSF agreed with the plasma concentrations in that the CTF levels in CSF were higher than those of ET-1 (see Fig. 22–1). Both ET-1 and CTF concentrations steadily increased after SAH, suggesting a correlation with the development of vasospasm. The absolute concentrations and the rates of increase in concentration, however, were markedly greater for CTF than for ET-1. Accordingly, the difference between basal and maximum values were 83 ±

TABLE 22–1 Clinical Characteristics of Five Patients with Hunt and Hess Grade 4 Subarachnoid Hemorrhage

Patient No.	Sex/Age (yrs)	Fisher Grade /Side	Localization of Aneurysm	Maximum CVS (%)	Clinical Presentation	Infarction	Clinical Outcome
1	F/42	3/bilateral	Anterior communicating artery	**74**	Generalized seizure	Multiple infarctions	Death
2	F/51	3/right	Right middle cerebral artery	**61**	Status epilepticus	No infarction; intraventricular hemorrhage	Death
3	M/45	3/bilateral	Anterior communicating artery	**52**	GCS4	Anterior cerebral artery	GCS12
4	F/60	3/bilateral	Anterior communicating artery	**65**	Seizure	Watershed infarction	GCS7
5	F/65	3/bilateral	Anterior communicating artery	**50**	GCS3	No infarction	GCS9

GCS, Glasgow coma score; CVS, cerebral vasospasm.

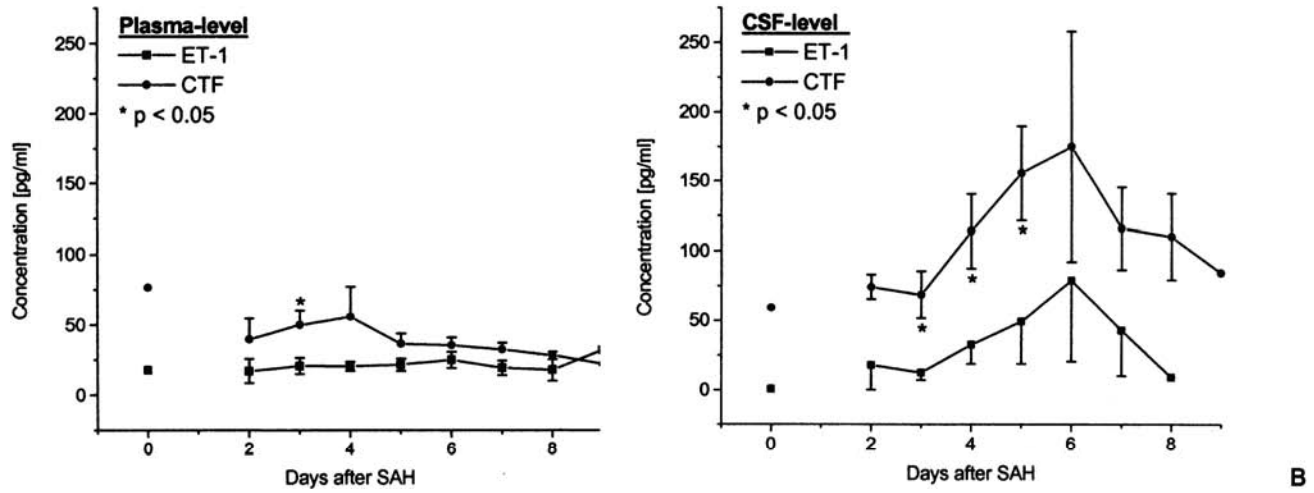


FIGURE 22–1 Concentration of endothelin (ET)-l and its companion peptide, C-terminal fragment (CTF), in plasma (A) and cerebrospinal fluid (CSF, B) in five patients with aneurysmal subarachnoid hemorrhage (SAH) and delayed cerebral vasospasm. Concentrations of CTF are higher in plasma and CSF compared with the respective ET-1 levels. In plasma only a mild increase of CTF occurred during the development of vasospasm, whereas there was a marked increase in CTF levels in CSF.

39 pg/mL for ET-1 and 154 ± 49 pg/mL for CTF. A significantly higher concentration of CTF compared with ET-1 occurred in CSF from 3 to 7 days after SAH. This increase was observed in patients with and without infarction, with a difference (basal vs maximum level) in CTF concentration in CSF being 183 ± 74 pg/mL in patients with infarction and 109 ± 12 pg/mL in patients without infarction.

Discussion adn Conclusion

Plasma concentrations of CTF displayed only moderate alterations after SAH without any obvious correlation with the development of vasospasm. This result is in agreement with previous observations that have suggested that plasma concentrations of ET-1 and big ET-1 do not correlate with the development of vasospasm.[7,9,16]

The markedly higher levels of CTF in CSF compared with ET-1 suggest that CTF is a more sensitive parameter for the activity of the cerebral ET system than ET-1 itself. This may be due to the clearance of at least a part of the ET-1 that is produced from the CSF by receptor binding and internalization.[15] CTF is generated in an equimolar fashion with ET-1, but there is no evidence for a receptor-associated clearance mechanism of CTF itself.[14] The maximum concentration of CTF in the CSF reached a level at which ET-1 would vasoconstrict human cerebral arteries.[17] Therefore, the present results further support an important role for ET-1 in the development of vasospasm and may help to explain prior findings that increased levels of ET-1 in CSF did not reach concentrations at which contraction of cerebral vessels in vitro would be expected.[7–9] Furthermore, the time of onset and the level of increase may be taken to suggest that CTF could be an indicator and even a predictor for the development of vasospasm after aneurysmal SAH. Further studies are under way to confirm the present results in a larger population.

REFERENCES

1. Feger GI, Schilling L, Ehrenreich H, Wahl M. Endothelin-induced contraction and relaxation of rat isolated basilar artery: effect of BQ-123. J Cereb Blood Flow Metab 1994;14:845–852
2. Papadopoulos SM, Gilbert LL, Webb RC, D'Amato CJ. Characterization of contractile responses to endothelin in human cerebral arteries: implications for cerebral vasospasm. Neurosurgery 1990;26:810–815
3. Zimmermann M, Seifert V, Loffler BM, Stolke D, Stenzel W. Prevention of cerebral vasospasm after experimental subarachnoid hemorrhage by RO 47–0203, a newly developed orally active endothelin receptor antagonist. Neurosurgery 1996;38:115–120
4. Wanebo JE, Arthur AS, Louis HG, et al. Systemic administration of the endothelin-A receptor antagonist TBC 11251 attenuates cerebral vasospasm after experimental subarachnoid hemorrhage: dose study and review of endothelin-based therapies in the literature on cerebral vasospasm. Neurosurgery 1998;43:1409–1418
5. Caner HH, Kwan AL, Arthur A, et al. Systemic administration of an inhibitor of endothelin-converting enzyme for attenuation of cerebral vasospasm following experimental subarachnoid hemorrhage. J Neurosurg 1996;85:917–922
6. Zimmermann M, Jung C, Raabe A, Spanehl O, Fach K, Seifert V. Inhibition of endothelin-converting enzyme activity in the rabbit basilar artery. Neurosurgery 2001;48:902–910
7. Seifert V, Loffler BM, Zimmermann M, Roux S, Stolke D. Endothelin concentrations in patients with aneurysmal subarachnoid hemorrhage: correlation with cerebral vasospasm, delayed

ischemic neurological deficits, and volume of hematoma. J Neurosurg 1995;82:55–62

8. Suzuki R, Masaoka H, Hirata Y, Marumo F, Isotani E, Hirakawa K. The role of endothelin-1 in the origin of cerebral vasospasm in patients with aneurysmal subarachnoid hemorrhage. J Neurosurg 1992;77:96–100

9. Ehrenreich H, Lange M, Near KA, et al. Long term monitoring of immunoreactive endothelin-1 and endothelin-3 in ventricular cerebrospinal fluid, plasma, and 24-h urine of patients with subarachnoid hemorrhage. Res Exp Med (Berl) 1992;192:257–268

10. Mascia L, Fedorko L, Stewart DJ, et al. Temporal relationship between endothelin-1 concentrations and cerebral vasospasm in patients with aneurysmal subarachnoid hemorrhage. Stroke 2001;32:1185–1190

11. Gaetani P, Rodriguez y Baena R, Grignani G, Spanu G, Pacchiarini L, Paoletti P. Endothelin and aneurysmal subarachnoid haemorrhage: a study of subarachnoid cisternal cerebrospinal fluid. J Neurol Neurosurg Psychiatry 1994;57:66–72

12. Pluta RM, Boock RJ, Afshar JK, et al. Source and cause of endothelin-1 release into cerebrospinal fluid after subarachnoid hemorrhage. J Neurosurg 1997;87:287–293

13. Rubanyi GM, Polokoff MA. Endothelins: molecular biology, biochemistry, pharmacology, physiology, and pathophysiology. Pharmacol Rev 1994;46:325–415

14. Turner AJ, Murphy LJ. Molecular pharmacology of endothelin converting enzymes. Biochem Pharmacol 1996;51:91–102

15. Wang J, Chiou WJ, Gagne GD, Wu-Wong JR. Internalization of type-A endothelin receptor. J Cardiovasc Pharmacol 2000; 36:S61–S65

16. Juvela S. Plasma endothelin and big endothelin concentrations and serum endothelin-converting enzyme activity following aneurysmal subarachnoid hemorrhage. J Neurosurg 2002; 97:1287–1293

17. Vatter H, Mursch K, Zimmermann M, et al. Endothelin-converting enzyme activity in human cerebral circulation. Neurosurgery 2002;51:445–452

23

Cytokines Produce Apoptosis in Cultured Cerebral Endothelial Cells

HITOSHI KIMURA, M.D., ILKER GULES, M.D.,
TOSHINARI MEGURO, M.D., JOHN H. ZHANG, M.D., PH.D.

Abstract

It has been established that the level of proinflammatory cytokines such as tumor necrosis factor-a (TNFa), interleukin-1ß (IL-1ß), IL-6, and IL-8 are elevated in cerebrospinal fluid of patients with subarachnoid hemorrhage. Cytokines in cerebrospinal fluid may contribute to the development of vasospasm and cerebral ischemia. This study was undertaken to investigate the possible cytotoxic effects of these cytokines on cultured cerebral microvascular endothelial cells. The effects of TNFa, IL-1ß, IL-6, and IL-8 were tested using cell viability assay, deoxyribonucleic acid (DNA) fragmentation analysis (DNA laddering), Western blot analysis [anti-poly(ADP-ribose) polymerase (PARP) antibody], and caspase-3 activity. A caspase-3 inhibitor was tested for its ability to attenuate the effect of cytokines. TNFa and IL-1ß, but not IL-6 or IL-8, caused cell detachment in a time- and dose-dependent manner ($p < .05$). TNFa (200 pg/mL) and IL-1ß (150 pg/mL) produced DNA ladders at 24 to 72 hours. TNFa but not IL-1ß cleaved PARP from a 116 kDa to a 85 kDa fragment at 24 to 72 hours after incubation. TNFa but not IL-1ß significantly enhanced caspase-3 activity at 24 to 72 hours. Caspase-3 inhibitors (10 µmol/L) significantly prevented TNFa-induced cell detachment compared with TNFa-treated cells ($p < .05$). TNFa induces apoptosis in cultured cerebral endothelial cells through the cleavage of caspase-3. IL-1ß decreases adherent cells and produces DNA ladders but fails to cleave PARP or increase caspase-3 activity. IL-1ß may induce apoptosis in cerebral endothelial cells through a different pathway from that of TNFa. Since elevated levels of TNFa and IL-1ß were reported in the bloody cerebrospinal fluid in patients of SAH, these actions of TNFa and IL-1ß may contribute to the pathogenesis of cerebral vasospasm.

Proinflammatory cytokines such as tumor necrosis factor-a (TNFa) and interleukin-1ß (IL-1ß) have been detected in the cerebrospinal fluid and serum of patients with subarachnoid hemorrhage (SAH).[1–5] Inflammatory genes are upregulated in dog basilar arteries removed during vasospasm after SAH.[6,7] Elevation of inflammatory cytokines may interfere with cerebral blood flow and aggravate cerebral ischemia.[8–10] In this study, we examined the cytotoxic effect of inflammatory cytokines on cultured cerebral microvascular endothelial cells.

Materials and Methods

Bovine brain microvascular endothelial cells were purchased from Cell Systems Corporation (Kirkland, WA, USA). Cell viability was determined by counting the number of adherent cells.[11,12] Cells were incubated with cytokines (TNFa and IL-lß) for 24, 48, or 72 hours. Recombinant rat TNFa and IL-1ß were purchased from R & D Systems, Inc (Gaithersburg, MD, USA). Genomic deoxyribonucleic acid (DNA) isolation was performed as specified by the manufacturer's protocol (Oncogene Research Products, Boston, MA, USA). The gel was then examined under ultraviolet light with ethidium bromide staining for visualization and photographing.[13,14] Proteolytic cleavage of poly(ADP-ribose) polymerase (PARP) was detected by Western blot analysis as previously described.[11,14] The caspase-3/CPP32 activity was measured with the use of a commercially available kit (Medical & Biological Laboratories, Nagoya, Japan) according to the manufacturer's instructions and the previous report.[11,13] Data are expressed as the mean ± the standard error of the mean. Statistical differences between the control and other groups were compared using one-way analysis of variance (ANOVA) and then the Tukey-Kramer multiple comparison procedure if a significant difference was found by ANOVA. A probability value (p value) of $< .05$ was considered statistically significant.

Results

Exposing endothelial cells to TNFa and IL-1ß caused marked cell detachment ($p < .05$) such that only 71% of cells were still attached after 72 hours incubation with 10 pg/mL TNFa and only 82% of cells were still attached after 72 hours incubation with 100 pg/mL IL-1ß. TNFa (200 pg/mL) produced DNA ladders and cleaved PARP from a 116 kDa to 85 kDa fragment after incubation with endothelial cells. PARP cleavage was suggested by reduction in the band at 116 and increase in the band at 85 kDa on Western blotting. IL-1ß (150 pg/mL) produced a DNA ladder after 72 hours incubation with endothelial cells, although exposure of cells to it was not associated with PARP cleavage. TNFa (200 pg/mL) induced 2.1,2.0, and 2.0-fold increases of caspase-3 activity after 24, 48, and 72 hours, respectively, which was significantly higher than the values from either nontreated cells or cells exposed to phosphate-buffered saline ($p < .05$). IL-1ß (150 pg/mL) did not affect caspase 3 activity when compared with nontreated cells ($p > .05$). Z-DEVD-FMK, a caspase-3 inhibitor, at 10 µmol/L significantly prevented TNFa-induced cell detachment, compared with TNFa-treated cells ($p < .05$).

Discussion

We have demonstrated that TNFa-induced apoptosis in cultured cerebral endothelial cells occurs through the activation of caspase 3. TNFa increased caspase 3 activity, which cleaves PARP, leads to DNA fragmentation, and results in cell detachment. Z-DEVD-FMK, a caspase-3 inhibitor, abolished the cytotoxic effect of TNFa. IL-lß produced a slight but significant decrease in the adherent cells mostly at 72 hours after incubation. The cytotoxic action of IL-1ß is independent of caspase 3 because IL-1ß produced DNA ladders without the activation of caspase 3 or cleavage of PARP

Astrocytes, endothelial cells, and neurons synthesize cytokines including TNFa and IL-1ß, particularly in response to various brain injuries including SAH.[15,16] Mathiesen et al reported that patients with SAH who had unfavorable outcome had an increase of TNFa levels (> 200 pg/mL) in the cerebrospinal fluid from 6 to 10 days after SAH.[3] The level of TNFa in patients who had good outcomes and the cerebrospinal fluid concentrations in normal controls were much lower. Nagata and colleagues reported that elevated concentrations of IL-1ß were present in the cerebrospinal fluid 3 (110 pg/mL) and 5 (156 pg/mL) days after severe SAH in humans.[17] We chose to use concentrations of TNFa and IL-1ß of ~200 pg/mL and 150 pg/mL, respectively, in most experiments in this study because these levels are similar to those observed in the cerebrospinal fluid of patients with unfavorable outcomes.[3,17]

TNFa and IL-1ß initiate pro-apoptotic pathways.[18,19] TNFa-induced receptor trimerization aggregates the death domains of the TNFa receptor I to form an active signaling complex. This active signaling complex can then recruit procaspase 8. Following activation, caspase 8 can initiate caspase-mediated cell death. Both caspase 8 and 9 can activate caspase 3 by proteolytic cleavage. Caspase 3 cleaves several substrates including PARP, lamins, and actins. This results in chromosomal DNA degradation and cellular morphological changes characteristic of apoptosis. Incubation of endothelial cells with TNFa markedly increases endothelial cell apoptosis via activation of caspase 3, a process that can be completely abrogated by inhibitors of caspases.[20] Our observation may have important clinical values because we used a concentration of TNFa (200 pg/mL) that is similar to the concentration reported in the cerebrospinal fluid of patients with SAH.[3]

IL-1ß activates nuclear factor KB and stress-activated protein kinases using a similar pathway initiated by ligand binding to the type-1 IL-1 receptor. IL-1ß plays an essential role in the activation of nuclear factor KB and cyclooxygenase-2 in cerebral endothelial cells.[21] However, TNFa and IL-1ß may have different roles in cerebral endothelial cells because, even though both are increased in cerebrospinal fluid after meningitis, it is TNFa that is related to damage to the blood-brain barrier (BBB) and to severity of disease.[22] Our observation indicates that if IL-1ß produces cell death by apoptosis, the mechanism is independent of the caspase-3 pathways This postulate is supported by the result shown here that IL-1ß did not activate caspase-3 and also failed to cleave PARP, a downstream effector of caspase-3. Endothelial cytotoxicity (apoptosis) induced by TNFa and IL-1ß would be expected to increase BBB permeability,[23,24] enhance cerebral vasospasm,[25] and worsen neurological injury after SAH.

Acknowledgment

This work was partially supported by a grant from the American Heart Association Bugher Foundation Award for Stroke Research and a grant from the National Institutes of Health (NS45694–01).

REFERENCES

1. Gruber A, Rossler K, Graninger W, Dormer A, Illievich MU, Czech T. Ventricular cerebrospinal fluid and serum concentrations of sTNFR-I, IL-1ra, and IL-6 after aneurysmal subarachnoid hemorrhage. J Neurosurg Anesthesiol 2000;12:297–306
2. Hirashima Y, Nakamura S, Endo S, Kuwayama N, Naruse Y, Takaku A. Elevation of platelet activating factor, inflammatory cytokines, and coagulation factors in the internal jugular vein of patients with subarachnoid hemorrhage. Neurochem Res 1997;22:1249–1255
3. Mathiesen T, Edner G, Ulfarsson E, Andersson B. Cerebrospinal fluid interleukin-1 receptor antagonist and tumor necrosis factor-alpha following subarachnoid hemorrhage. J Neurosurg 1997;87:215–220
4. Nam DH, Kim JS, Hong SC, et al. Expression of interleukin-1 beta in lipopolysaccharide stimulated monocytes derived from patients with aneurysmal subarachnoid hemorrhage is correlated with cerebral vasospasm. Neurosci Lett 2001;312:41–44
5. Osuka K, Suzuki Y, Tanazawa T, et al. Interleukin-6 and development of vasospasm after subarachnoid haemorrhage. Acta Neurochir (Wien) 1998;140:943–951
6. Aihara Y, Kasuya H, Onda H, Hori T, Takesda J. Quantitative analysis of gene expressions related to inflammation in canine spastic artery after subarachnoid hemorrhage. Stroke 2001; 32:212–217
7. Onda H, Kasuya H, Takakura K, et al. Identification of genes differentially expressed in canine vasospastic cerebral arteries after subarachnoid hemorrhage. J Cereb Blood Flow Metab 1999;19:1279–1288
8. Fassbender K, Hodapp B, Rossol S, et al. Inflammatory cytokines in subarachnoid haemorrhage: association with abnormal blood flow velocities in basal cerebral arteries. J Neurol Neurosurg Psychiatry 2001;70:534–537
9. Peterson JW, Kwun BD, Hackett JD, Zervas NT. The role of inflammation in experimental cerebral vasospasm. J Neurosurg 1990;72:767–774
10. Peterson JW, Kwun BD, Teramura A, et al. Immunological reaction against the aging human subarachnoid erythrocyte: a model for the onset of cerebral vasospasm after subarachnoid hemorrhage. J Neurosurg 1989;71:718–726
11. Meguro T, Chen B, Parent AD, Zhang JH. Caspase inhibitors attenuate oxyhemoglobin-induced apoptosis in endothelial cells. Stroke 2001;32:561–566
12. Meguro T, Klett CP, Chen B, Parent AD, Zhang JH. Role of calcium channels in oxyhemoglobin-induced apoptosis in endothelial cells. J Neurosurg 2000;93:640–646
13. Meguro T, Chen B, Lancon J, Zhang JH. Oxyhemoglobin induces caspase-mediated cell death in cerebral endothelial cells. J Neurochem 2001;77:1128–1135
14. Ogihara K, Zubkov AY, Bernanke DH, Lewis AI, Parent AD, Zhang JH. Oxyhemoglobin-induced apoptosis in cultured endothelial cells. J Neurosurg 1999;91:459–465
15. Chyatte D. Anti-inflammatory agents and cerebral vasospasm. Neurosurg Clin N Am 1990;1:433–450
16. Sercombe R, Dinh YR, Gomis P. Cerebrovascular inflammation following subarachnoid hemorrhage. Jpn J Pharmacol 2002;88:227–249
17. Nagata K, Sasaki T, Iwama J, et al. Failure of FK-506, a new immunosuppressant, to prevent cerebral vasospasm in a canine two-hemorrhage model. J Neurosurg 1993;79:710–715
18. Haridas V, Darnay BG, Natarajan K, Heller R, Aggarwal BB. Overexpression of the p80 TNF receptor leads to TNF-dependent apoptosis, nuclear factor-kappa B activation, and c-Jun kinase activation. J Immunol 1998;160:3152–3162
19. Mallat Z, Tedgui A. Apoptosis in the vasculature: mechanisms and functional importance. Br J Pharmacol 2000;130:947–962
20. Dimmeler S, Haendeler J, Rippmann V, Nehls M, Zeiher AM. Shear stress inhibits apoptosis of human endothelial cells. FEBS Lett 1996;399:71–74
21. Laflamme N, Lacroix S, Rivest S. An essential role of interleukin-lbeta in mediating NF-kappaB activity and COX-2 transcription in cells of the blood-brain barrier in response to a systemic and localized inflammation but not during endotoxemia. J Neurosci 1999;19:10923–10930
22. Sharief MK, Ciardi M, Thompson EJ. Blood-brain barrier damage in patients with bacterial meningitis: association with tumor necrosis factor-alpha but not interleukin-1 beta. J Infect Dis 1992;166:350–358
23. de Vries HE, Blom-Roosemalen MC, van Oosten M, et al. The influence of cytokines on the integrity of the blood–brain barrier in vitro. J Neuroimmunol 1996;64:37–43
24. Megyeri P, Abraham CS, Temesvari P, Kovacs J, Vas T, Speer CP. Recombinant human tumor necrosis factor alpha constricts pial arterioles and increases blood-brain barrier permeability in newborn piglets. Neurosci Lett 1992;148:137–140
25. Zubkov AY, Ogihara K, Bernanke DH, Parent AD, Zhang J. Apoptosis of endothelial cells in vessels affected by cerebral vasospasm. Surg Neurol 2000;53:260–266

SECTION IV

Experimental—Pathophysiology

24

Controversial Issues Regarding the Pathophysiology of Vasospasm: A Review

SHIGERU NISHIZAWA, M.D., PH.D.

Many papers have been published attempting to clarify the pathophysiological mechanisms underlying vasospasm after subarachnoid hemorrhage (SAH). Although the papers reached rational conclusions, there is still no general consensus on a variety of controversial issues regarding the pathophysiological mechanisms of vasospasm after SAH. This chapter highlights some of these controversial issues [specifically the role of the Rho/Rho kinase system and of protein kinase C (PKC) in vasospasm] and clarifies some of the questions that still need answers.

Rho/Rho Kinase and Myosin Light Chain Phosphorylation in Vasospasm

Phosphorylation of myosin light chain 20 (MLC20) has been considered an essential step in the contraction of cerebrovascular smooth muscle. During development of tonic tension in smooth muscle, phosphorylation of MLC20 is enhanced.[1] However, the increase in phosphorylation of MLC20 declines to basal or near-basal levels during the sustained phase of contraction.[1] This creates a discrepancy between contraction and phosphorylation of MLC20. Because vasospasm is in large part a sustained contraction of smooth muscle, the extent to which MLC20 remains phosphorylated during vasospasm has been studied, with somewhat conflicting results.[2] Recently, however, additional mechanisms involved in sustained smooth muscle contraction have been described, and the role of MLC20 has been reevaluated from the viewpoint of one of these systems, the Rho/Rho kinase system.[3–6] Rho is a low molecular weight monomeric G protein that is involved in activation of an effector protein, Rho kinase. In tonic smooth muscle contraction, Rho/Rho kinase not only enhances phosphorylation of MLC20 but also phosphorylates MLC phosphatase. This phosphorylation of MLC phosphatase significantly inhibits its function, resulting in continuation of myosin–actin interaction in the presence of reduced levels of MLC20 phosphorylation.[2–5] This phenomenon is termed Ca^{2+} sensitization because, in addition to reduced levels of MLC20, contraction persists despite a decline in intracellular Ca^{2+} concentrations. In addition, in this situation a long-lasting and relatively low level of phosphorylation of MLC20 continues under conditions of low energy consumption, and this situation can be considered to be an important candidate for the mechanism of vasospasm. Support of this mechanism includes the data from several investigators reporting that the Rho kinase specific inhibitor Y-27632 significantly inhibits vasospasm when applied topically to the vasospastic basilar artery that had been exposed via a transclival approach after SAH in dogs.[4,7] It also has been reported that oxyhemoglobin-induced tonic contraction of rabbit basilar artery in isometric tension in vitro is inhibited by Y-27632.[8]

On the other hand, the opposite result has also been reported, highlighting a problem with consistency in the vasospasm literature.[9] This report describes the relation between the roles of Rho kinase and PKC isoforms. Treatment of vasospasm in vivo by cisternal injection of Y-27632 showed that Y-27632 inhibited the initiation of vasospasm but not the maintenance of vasospasm in dogs. Y-27632 inhibited the phosphorylation of MLC20 and translocation of PKC d but not PKC a. From these results, the authors concluded that Rho kinase initiates the development of vasospasm through PKC d activity but that the maintenance of vasospasm was dependent on the activation of PKC a. In addition, high phosphorylation levels of MLC20 remained for more than 2 weeks, at which time the

diameter of vasospastic basilar artery had returned to normal. Histological studies suggest that alterations in smooth muscle (attributed to a phenotypic switch or change of smooth muscle, albeit without rigorous scientific data to support the contention) start to occur 1 week after SAH and continue for more than 2 weeks.[10]

Contractility of vasospastic arteries from a variety of cerebral arteries in experimental studies in response to maximal stimulation with high K^+ solution declines with increasing time after SAH.[2,11] According to these studies, the roles of Rho kinase and phosphorylation of MLC20 in the mechanism of vasospasm remain unclear. An explanation for vasospasm that relies solely on activation of Rho kinase and phosphorylation of MLC20 would suggest that all of the arterial diameter reduction in vasospasm would be due to active myogenic tone. However, as already described, histological changes in vasospastic arteries occur within a week of SAH, and contractility of vasospastic cerebral arteries diminishes significantly within this time.[10,11] This raises several questions. Is it really possible to explain the mechanism of vasospasm based on high levels of MLC20 phosphorylation and/or on Rho kinase–induced Ca^{2+} sensitization? What is the explanation for such contradictory experimental results? Are they due to different experimental methods employed or to some other as yet undefined factor?

Protein Kinase C and Vasospasm

Many publications have addressed the role of PKC in vasospasm after SAH.[12–18] Early research showed that tonic cerebral vascular contraction was induced by phorbol esters in an isometric tension study.[11]

Phorbol esters are potent activators of PKC. Thereafter, research was conducted to identify whether PKC activity was enhanced after SAH and to define whether the activation coincided with the time course of experimental vasospasm. Since it was first shown that a significant translocation of PKC from the cytosolic to the membrane fraction of arterial extracts occurred during the time course of vasospasm, it has been confirmed that activation of PKC, which is associated with such a translocation, plays a role in the mechanism of vasospasm.[13]

There are at least two theories regarding the role of PKC. One is that PKC itself induces tonic cerebral vascular contraction, and the second is that the mechanism involves the augmentation of MLC phosphorylation through CPI-17 (PKC-potentiated phosphatase inhibitory protein of 17 kDa molecular weight). In this mechanism, PKC activates CPI-17, which has a similar effect to that of Rho kinase. The CPI-17 phosphorylates MLC phosphatase, resulting in the inhibition of MLC phosphatase and inducing a long-lasting high phosphorylation of MLC20.[8,15] It is apparent from the preceding discussion that this would result in another situation where there would be Ca^{2+} sensitization.

As described in the previous section discussing Rho kinase, there has been evidence presented that the degree of vasospasm and the level of phosphorylation of MLC20 are not correlated.[14] Furthermore, inhibitors of PKC-specific isoforms suppressed vasospasm in a dog model despite persistence of high levels of MLC20 phosphorylation. Based on these data, it has been concluded that PKC plays a role in the mechanism of vasospasm and induces tonic cerebral vascular contraction.[14] However, what has not been clarified is the mechanism by which PKC accomplishes this. Questions remaining also include how PKC sustains the chronic phase of vasospasm. One possibility is that the mechanism relates to actin or another thin-filament-related process that is involved in contraction.

Another controversy concerns which isoforms of PKC play a role in the mechanism of vasospasm. In the first published paper, PKC a and e were detected in canine basilar artery and reported to have significant roles in the mechanism of vasospasm in the double-hemorrhage canine model.[16] In this paper, the samples were not crude, but purified. The expression of these two PKC isoforms was found to be decreased as the vasospasm progressed. It was concluded that PKC was consumed and downregulated because PKC was upregulated at some period of time during the course of vasospasm. A more recent study reported that cultured smooth muscle cells from rabbit basilar artery expressed PKC a and e and that this expression was enhanced by oxyhemoglobin, a prime candidate of for the spasmogenic substance causing vasospasm.[8] Therefore, it was concluded that these isoforms were important in the development of vasospasm.

On the other hand, we had already shown that four PKC isoforms (PKC a, d, ., and .) were expressed in canine basilar arteries and that only PKC a and d were altered in chronological fashion as vasospasm progressed.[17,18] Initially, PKC d was translocated to the membrane fraction, and PKC a translocation followed in the chronic phase of vasospasm. The PKC isoform–specific antibodies were derived from rat brain but specificity of the antibodies to PKC isoform bands was confirmed using Western blotting with positive controls from rat brain and by blocking peptides specific to the PKC isoforms.[17,18] An experimental study in vivo also showed that the PKC δ-specific inhibitor rottlerin inhibited the initiation of vasospasm, but not the maintenance of vasospasm, whereas a classical/novel PKC inhibitor, chelerythrine, inhibited the entire course of vasospasm in the two-hemorrhage

canine model.[19,20] From these results, we concluded that PKC δ plays a role in the initiation of and PKC α in the maintenance of vasospasm.[14,17,18] The results discussed here, however, highlight the remaining controversy as to which PKC isoforms are involved in the mechanism of vasospasm. The published papers use different methods and experimental animals. The explanation remains to be clarified.

The author suggests that subsequent experiments consider the following points. If it is to be concluded that PKC is important in the mechanism of vasospasm, then upregulation of some aspect of PKC (protein, function, translocation, or other method) should be shown at some point during the course of vasospasm. If a PKC isoform is concluded to play an important role in the mechanism of vasospasm, the specificity of the expressed PKC isoform has to be shown. Data showing only positive controls using tissues from other species do not adequately prove that a specific isoform is altered. Because the time course of clinical vasospasm spans a few weeks, short-term experiments in vitro (e.g., lasting 1–2 hours) may not provide data relevant to the mechanism of vasospasm. Such results might relate to the mechanism of the initiation of vasospasm but would be unlikely to reveal mechanisms related to the maintenance of vasospasm. Furthermore, care should be taken to conclude that a mechanism is important in vasospasm based on experiments using isolated spasmogenic substances in vitro. Spasmogenic substances causing vasospasm probably are numerous and include many compounds and reactions in addition to oxyhemoglobin, so that results in vitro that use one species unrelated to humans and one spasmogen may produce results that are not important to the mechanism of vasospasm after SAH in humans.

REFERENCES

1. Driska SP, Aksoy MO, Murphy RA. Myosin light chain phosphorylation associated with contraction in arterial smooth muscle. Am J Physiol 1981;240:C222–C233
2. Macdonald RL, Weir B. Cerebral Vasospasm. New York: Academic; 2001
3. Kitazawa T, Kobayashi S, Horiuti K, Somlyo AV, Somlyo AP. Receptor-coupled, permeabilized smooth muscle: role of the phosphatydil inositol cascade, G-protein and modulation of the contractile response to Ca^{2+}. J Biol Chem 1989;264:5339–5342
4. Uehata M, Ishizaki T, Satoh H, et al. Calcium sensitization of smooth muscle mediated by a Rho-associated protein kinase in hypertension. Nature 1997;389:990–994
5. Somlyo AP, Somlyo AV. Signal transduction by G-proteins, rho-kinase and protein phosphatase to smooth muscle and nonmuscle myosin II. J Physiol 2000;522:177–185
6. Nobe K, Paul RJ. Distinct pathways of Ca^{2+} sensitization in porcine coronary artery: effects of Rho-related kinase and protein kinase C inhibition on force and intracellular Ca^{2+}. Circ Res 2001;88:1283–1290
7. Sato M, Tani E, Fujikawa H, Kiabuchi K. Involvement of Rho-kinase-mediated phosphorylation of myosin light chain in enhancement of cerebral vasospasm. Circ Res 2000;87:195–200
8. Wickman G, Lan C, Vollrath B. Functional roles of the Rho/Rho kinase pathway and protein kinase C in the regulation of cerebrovascular constriction mediated by hemoglobin: relevance to subarachnoid hemorrhage and vasospasm. Circ Res 2003;92:809–816
9. Nishizawa S, Koide M, Obara K, Nakayama K, Yamaguchi M. Protein kinase C isoforms, rho-kinase and myosin light chain phosphorylation as a mechanism of cerebral vasospasm after subarachnoid hemorrhage. In: Macdonald RL, ed. Cerebral Vasospasm: Advances in Research and Treatment. New York: Thieme Medical Publishers; 2004;36–40
10. Yamaguchi M, Nishizawa S, Koide M, Nonaka Y. Smooth muscle phenotype change in canine basilar artery following subarachnoid hemorrhage. In: Macdonald RL, ed. Cerebral Vasospasm: Advances in Research and Treatment. New York: Thieme Medical Publishers; 2004;62–64
11. Koide M, Nishizawa S, Ohta S, Yokoyama T, Namba H. Chronological change of contractile mechanism in prolonged vasospasm after subarachnoid hemorrhage: from protein kinase C to tyrosine kinase. Neurosurgery 2002;51:1468–1476
12. Nishizawa S, Peterson JW, Shimoyama I, Uemura K. Relation between protein kinase C and calmodulin systems in cerebrovascular contraction: investigation of the pathogenesis of vasospasm after subarachnoid hemorrhage. Neurosurgery 1992;31:711–716
13. Nishizawa S, Nezu N, Uemura K. Direct evidence for a key role of protein kinase C in the development of vasospasm after subarachnoid hemorrhage. J Neurosurg 1992;76:635–639
14. Nishizawa S, Obara K, Koide M, et al. Specific attenuation of canine vasospasm after subarachnoid hemorrhage by protein kinase C inhibitors despite augmented phosphorylation of myosin light chain. J Vasc Res 2003;40:169–178
15. Laher I, Zhang J. Review article: protein kinase C and cerebral vasospasm. J Cereb Blood Flow Metab 2001;21:887–906
16. Takuwa Y, Matsui T, Abe Y, et al. Alterations in protein kinase C activity and membrane lipid metabolism in cerebral vasospasm after subarachnoid hemorrhage. J Cereb Blood Flow Metab 1993;13:409–415
17. Nishizawa S, Obara K, Nakayama K, et al. Protein kinase C a and δ are involved in the development of vasospasm after subarachnoid hemorrhage. Eur J Pharmacol 2000;398:113–119
18. Nishizawa S, Obara K, Nakayama K, et al. Which protein kinase C isoforms are involved in the development of vasospasm after subarachnoid hemorrhage? Acta Neurochir Suppl 2001;77:21–24
19. Gschwendt M, Muller HJ, Kielbassa K, et al. Rottlerin, a novel protein kinase inhibitor. Biochem Biophys Res Commun 1994;199:93–98
20. Herbert JM, Augereau JM, Gleye J, Maffrand JP. Chelerythrine is a potent and specific inhibitor of protein kinase C. Biochem Biophys Res Commun 1990;172:993–999

25

Cerebral Vasospasm Revisited: SAH Syndrome

TOMIO OHTA, M.D., PH.D.

A half century has passed since Ecker and Riemenschneider demonstrated angiographic cerebral vasospasm in clinical cases.[1] In Japan, we reported the first case of cerebral vasospasm in 1963, 12 years after their report. Cerebral angiography was performed in a 22-year-old medical student, whose aneurysm was located at the trifurcation of the middle cerebral artery. He suffered from delayed ischemic deficits several days after subarachnoid hemorrhage (SAH). It was recommended that he undergo surgery to clip the aneurysm, but he and his family rejected this advice. He died 6 months later of recurrent SAH. Since then, the annual meeting of cerebral vasospasm has been called Spasm Symposium in Kyoto, which was founded in 1985. The main theme of the first symposium addressed whether cerebral vasospasm is really spasm. Annual proceedings have been published in Japanese and include the work of distinguished guest speakers invited from abroad. This chapter reviews the author's personal experimental studies and clinical experiences as they relate to the problem of cerebral vasospasm.

Experimental Studies

Severity of Vasospasm

One of the most worrisome aspects of experiments on cerebral vasospasm is the severity of cerebral vasospasm produced in the model that is utilized. The most obvious fact to note is that mild or moderate vasospasm demonstrated by cerebral angiography gives rise to little clinical concern because it does not generally cause any ischemic symptoms. On the other hand, severe, diffuse vasospasm produces delayed cerebral ischemia. Therefore, severe vasospasm should be used in the experimental study of vasospasm, whether this be in animal models or when studying the effects of vasodilators on vasoconstrictions obtained in vitro. We have reported that when $BaCl_2$ solution was applied to the rat basilar artery, the artery became narrowed and threadlike. Some branches became pale and faded due to lack of blood flow (Fig. 25–1). In this series of exper-

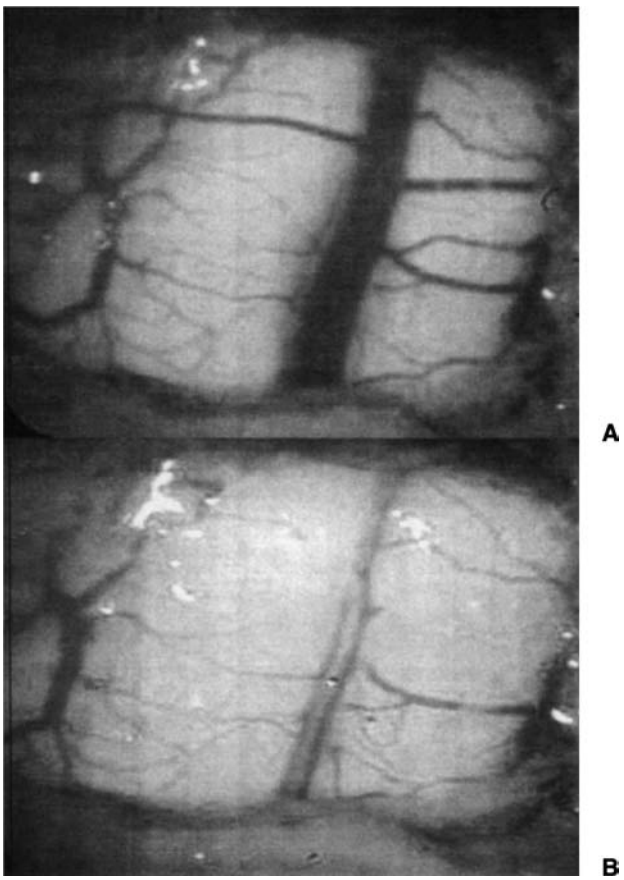


FIGURE 25–1 Photographs of the rat basilar artery exposed transclivally. The normal appearance and diameter of the basilar artery (A) is compared with the appearance after topical application of barium chloride solution (B), which produces severe constriction of the artery.

iments we observed severe histological changes in the spastic vessel wall.[2] Furthermore, when the artery was maintained in a spastic state for more than 3 hours, it was noted that the spasm could not be reversed with papaverine hydrochloride, as has been possible in some clinical cases.[2]

Biphasic Vasospasm and Vasodilatation

Although there is some discussion as to the existence of acute vasospasm after SAH in humans, neither biphasic vasospasm nor biphasic postspastic vasodilatation has been of great interest in clinical cases. Biphasic vasospasm has, however, been the focus of experimental studies. During the process of washing $BaCl_2$ solution away from the rat basilar artery with saline solution, we often observed vasodilatation at either or both the trunk and its branches, immediately and 20 to 30 minutes after washing. This vasodilatation accompanied by vasospasm would have been observed more frequently in clinical cases if we had known of the existence of this phenomenon (Fig. 25–2).

New Model *for Pharmacological Study*

Another important consideration is the experimental model used for the pharmacological study of vasospasm. Helical strips or ring preparations are widely adopted for this purpose, but they are not useful for testing the specific effects of vasoactive agents that are applied specifically to the luminal or abluminal aspect of blood vessels or for testing different agents applied to both sides simultaneously. From the clinical point of view, a system that can separate extra- and intraluminal aspects of blood vessels is, in the view of this author, mandatory because, for the cerebral arteries, there exists an extraluminal route of drug administration unlike that for other blood

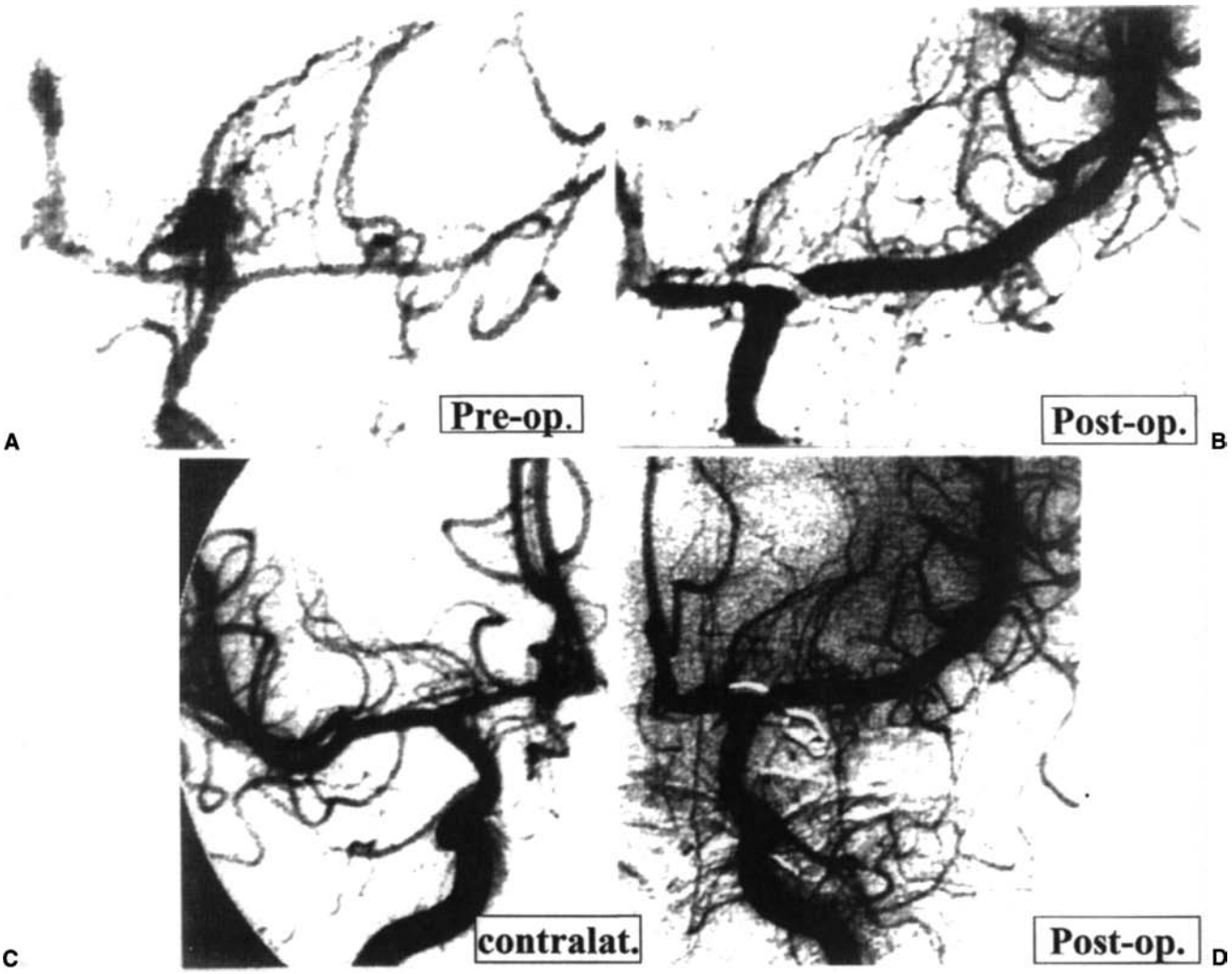


FIGURE 25–2 Photographs of anteroposterior cerebral angiograms of the left internal carotid artery taken before (A) and after surgery (B). Comparison of the postoperative left carotid angiogram (D) to the angiogram of the right side taken before surgery (C) shows that the left middle cerebral artery appears dilated after the resolution of vasospasm.

vessels. We have developed a constant flow perfusion system with which the vascular responses to vasoactive agents, applied either extra- or intraluminally, can be measured via a differential pressure gauge. Furthermore, different vasoactive agents can be applied in combination on both sides.

We have observed with this perfusion system the impressive reaction of the cerebral artery to KCl solution when it is applied intraluminally whereas when it is applied extraluminally the artery does not react. No such differences in contractile activity are observed when serotonin is applied to either aspect of the vessel.[3] Also, we have found that there was no difference between extra- and intraluminal application of endothelin, although there are conflicting reports that endothelin acts from the abluminal or from the luminal side.[4]

Permeability Change of Major Cerebral Arteries in Experimental Vasospasm

The permeability of spastic cerebral arteries was examined using horseradish peroxidase (HRP) in the double-injection model of SAH in dogs. The distribution of HRP was assessed by transmission electron microscopy There was a substantial amount of HRP in the intermuscular space in the tunica media when HRP was injected intracisternally, whereas no penetration of HRP into the vessel wall was observed when HRP was injected intravenously. These results suggest that spasmogenic substances may penetrate the vessel wall from the extraluminal side more easily. This would be possible for compounds with a molecular size equal to or less than HRP, although the penetration of larger molecules was not assessed in this study.[5]

Experimental Study of Pathological Arteries Affected by Atherosclerosis, Hypertension, or Diabetes Mellitus

In addition to vasospasm, we are very interested in proliferative angiopathies. The author believes that functional vasoconstriction would not maintain cerebral arterial narrowing in vasospasm for days and that there must be some accompanying structural change in the vasospastic arterial wall. The observation that endovascular dilatation with balloon techniques of the proximal vasospastic cerebral arteries results in long-lasting dilation is suggested to be evidence for the existence of such structural or organic changes. Atheromatous plaques are commonly seen in the arterial walls around the circle of Willis, and there are many publications on the effects of platelet derived growth factor on smooth muscle cell migration into the subendothelial space of atherosclerotic arteries.[6] To identify the specific reaction of an atherosclerotic artery to SAH, Kurosawa and Kusanagi hypercholesterolemic rabbits were used to study vasospasm after SAH.[7] These rabbits have a hereditary deficiency of low-density lipoprotein receptors. They develop typical atheromatous plaques in the larger systemic arteries but not the cerebral arteries. Therefore, a vasospasm model was developed where the common carotid artery on one side was punctured with a needle. The artery was surrounded with a silicone tube so that blood was maintained in the periadventirial area, mimicking the manner in which blood surrounds the cerebral arteries after SAH. Although the magnitude of maximal narrowing did not differ from that occurring in control rabbits, the spasm lasted for a significantly longer time (7 days). Also, the degee of vasodilatation with papaverine hydrochloride injected into the common carotid artery of hypercholesterolemic rabbits was significantly less than in controls. These results are indicative of structural changes in the atherosclerotic arteries.[8]

Role of White Thrombi in Delayed Ischemic Neurologica Deficits

The author worked at the National Institutes of Health in the United States as a visiting scientist from 1963 to 1966. He exposed the arteries around the circle of Willis in cats by splitting the corpus callosum and operating through the ventricular system. Using this method, a segment of cerebral artery was constricted by topical application of serotonin. Histological examination of this segment demonstrated intraluminal white thrombi, which appeared white under the operating microscope (Fig. 25–2).[9] At that time, Francis Echlin had performed experiments on the cerebral arteries of monkeys that were exposed by craniotomy and opening of the arachnoid membrane. Echlin reported that severe vasospasm could be produced by mechanical stimuli and by topical application of whole blood.[10] A photo shown in Echlin's publication showed a similar appearance to this author's own observations, although Echlin did not refer to the histological confirmation.

Recently the frequency of occurrence of cerebral vasospasm has been reduced, possibly in part because clipping of aneurysms is performed under the operating microscope by skillful neurosurgeons. There also are reports that vasospasm is less common after endovascular treatment of aneurysms with Guglielmi detachable coils than after craniotomy and clipping. One interpretation of this information is that vasospasm occurs more easily when there is mechanical manipulation of the cerebral arteries in addition to SAH. We reported the role of mechanical stimuli in the occurrence of cerebral vasospasm.[9]

Clinical Experiences

Timing of Computed Tomographic Scanning after SAH

The relationship between severe vasospasm and the severity of SAH shown on computed tomography (CT) has been widely accepted. However, caution is needed in evaluating the extent of SAH on CT images. In some cases, we have performed serial CT scanning before surgery and noted that there may be almost complete clot clearance within 24 hours of SAH in the absence of surgery.[11]

A Special Case Experience

Recently we have reported an interesting case of vasospasm. A 23-year-old man complained of dysphasia and right hemiparesis 2 weeks after SAH. Magnetic resonance imaging revealed a small amount of high-signal hematoma around the internal carotid artery on the left side, and an ill-defined infarction of the left frontal operculum. Cerebral angiograms showed an aneurysm at the bifurcation of the left internal carotid artery. Severe vasospasm was demonstrated in the supraclinoid segment of the internal carotid artery and the proximal portions of the anterior and middle cerebral arteries. Three weeks after SAH, an elective craniotomy was performed. The aneurysm was successfully clipped. The appearance of the parent artery that had been shown to be vasospastic angiographically was unusual. There was no vasospasm of the distal internal carotid or of the proximal segments of the anterior and middle cerebral arteries. The arteries were rather expansive and appeared and felt like dilated veins. Manipulation of the arteries at surgery gave the impression that they were abnormally soft and thin-walled. The dilation was confirmed by postoperative cerebral angiography that was performed to document complete clipping of the aneurysm (Fig. 25–3).

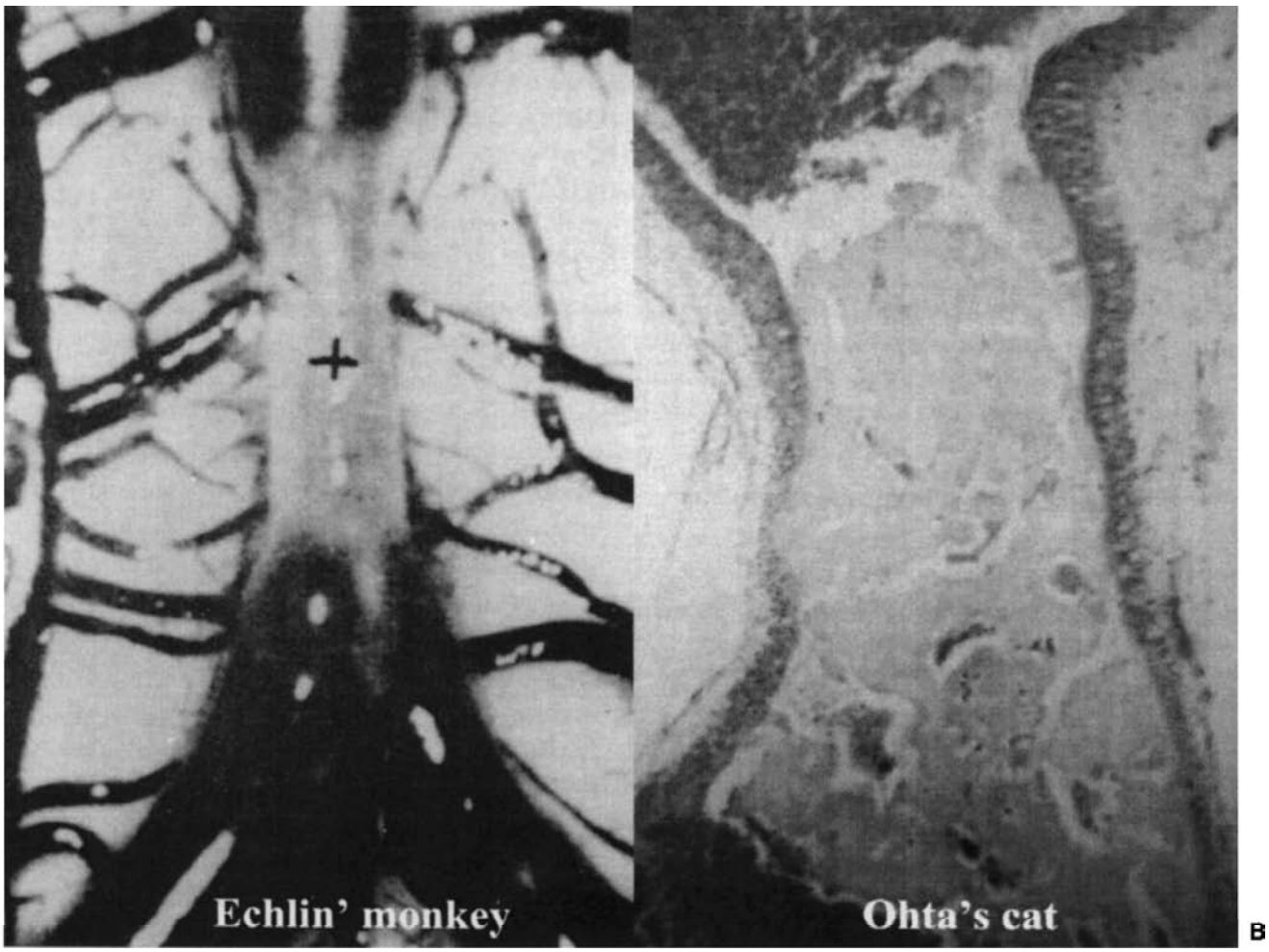


FIGURE 25–3 Photographs of the spastic monkey basilar artery observed in Echlin's experiments (left). The pale portion (under the cross) may be occupied by white thrombus, as suggested by the photomicrograph of the spastic cat middle cerebral artery shown on the right (hematoxylin and eosin).

A Hypothesis of Two Types of Cerebral Vasospasm

The preceding case caused this author to speculate that there might be two kinds of vasospasm following SAH: one being the spasm seen in relatively young persons whose cerebral arteries are otherwise healthy, the other occurring in elderly patients whose arteries are atherosclerotic and contain smooth muscle cells already exposed to various kinds of proliferative stimuli. Although arterial narrowing seen in the former patients is mainly caused by active smooth muscle contraction that is a functional vasospasm, the vasospasm in older patients is a passive organic angiopathy that occurs because of preexisting structural changes such as atherosclerosis. In addition, because atherosclerotic changes are predominantly in the basal arterial trunks rather than in the peripheral cerebral arteries, there might be an occasion for coexistence of these two types of vasospasm in a single arterial tree with passive organic angiopathy in the proximal atherosclerotic segments and active functional vasospasm in the peripheral ones. Therefore, endovascular angioplasty and intraarterial infusion of effective vasodilating agents should be applied in combination in cases of cerebral vasospasm.

Treatment Strategy Including Instantaneous Selective Brain Cooling

In addition to the search for basic treatments to prevent vasospasm, delayed ischemic neurological deficits should be identified as soon as possible so that we can initiate reasonable treatment within a therapeutic time window. Recently we have developed a new, less invasive method to induce hypothermia, which we call instantaneous selective brain cooling. This is at present an experimental treatment. Cold Ringer's solution is perfused through the vertebral artery and cool venous blood from the brain is withdrawn from the jugular vein and returned to the inferior vena cava through a dialyzer. With this method, a brain temperature of 32°C can be obtained within several minutes, while the rectal temperature remains within the normal range.[12] In addition, one cerebral hemisphere can be cooled by inserting the tip of the catheter into the ipsilateral internal carotid artery via transfemoral catheterization. Less invasive and instantaneous hypothermia in addition to bloodless surgery will be possible in the near future.

Summary

The incidence of cerebral vasospasm has decreased in recent years in part due to improvement in operative techniques and the advent of endovascular coiling of aneurysms. Research is still needed, however, to eliminate the problem of vasospasm. Experiments should faithfully reproduce the clinical signs and symptoms of cerebral vasospasm. These include:

1. The severity of the experimental vasospasm should be similar to that of clinical cases.
2. The irreversibility of clinical vasospasm in response to many vasodilating agents might suggest that vasospasm consists not only of functional but also of organic components, as discussed earlier.
3. With regard to experimental animal models, the study of SAH in animals with hypercholesterolemia, hypertension, and/or diabetes mellitus should be considered.

As for treatment of cerebral vasospasm, two types of vasospasm are suggested. These two types suggest that both intra-arterial infusion of vasodilating agents and endovascular angioplasty should be used in combination. In the near future, instantaneous brain cooling would be applicable to protect the brain from ischemia within a reasonable therapeutic time window.

Conclusion

The results of several decades studying vasospasm have led this author to refer to vasospasm as an SAH syndrome. It is part of Japanese culture and the essence of Japanese ancestral philosophy to consider everything from both sides. Cerebral vasospasm should be considered from both experimental and clinical observations. A theory, observation, or treatment effect may be incorrect even though it seems reasonable when considered from only the experimental or the clinical side.

Cerebral vasospasm presents an ongoing challenge for neurosurgeons in both the clinical and the experi mental realms. Experimental studies are often in re sponse to ideas that arise from clinical cases, and in turn the fruits of the research enable neurosurgeons to better help patients that follow. Maintaining a clinical and experimental balance allows the natural fruition of reasonable and practical ideas. It is this author's wish to impart the wisdom of this balance to a new generation of nurosurgens.

Acknowledgments

I would like to express my sincere gratitude to Professor R. Loch Macdonald, who served as president of the 8th International Conference on Cerebral Vasospasm and who gave me the great privilege of attending this wonderful conference in Chicago as an honored guest. I also thank Professor Mario Zuccarello for help

preparing this manuscript and Dr. Hiroshi Hasegawa, vice director of our hospital, for his thoughtful advice for the manuscript.

REFERENCES

1. Ecker A, Riemenschneider P. Arteriographic demonstration of spasm of the intracranial arteries: with special reference to saccular arterial aneurysm. J Neurosurg 1951;8:660–667
2. Ohta T, Kajikawa H, Funatsu N, Yoshikawa Y, Someda K. Cerebral vasospasm and its relaxation responses to vasodilators: pathological study of severe prolonged vasospasm. In: Wilkins RH, ed. Cerebral Arterial Spasm. Baltimore, MD: Williams and Wilkins; 1980:132–138
3. Ohta T, Mori R, Ogawa R, Tsuji M. Development of a new perfusion system for pharmacological study on rabbit basilar arteries. Stroke 1991;22:384–389
4. Kazuki S, Ohta T, Ogawa R, et al. Effect of intraluminal or extralu–minal endothelin on perfused rabbit basilar arteries. J Neurosurg 1997;86:859–865
5. Ohta T, Sato G, Kuroiwa T. The permeability change of major cerebral arteries in experimental vasospasm. Neurosurgery 1992;30:331–336
6. Boucher P, Gotthardt M. LRP and PDGF signaling: a pathway to atherosclerosis, Trends Cardiovasc Med 2004;14:55–60
7. Kurosawa T, Kusanagi M, Yamasaki Y, Senga Y, Yamamoto T. New mutant rabbit strain with hypercholesterolemia and atherosclerotic lesions produced by serial inbreeding. Lab Anim Sci 1995;45:385–392
8. Ohta T. Role of an atherosclerosis to "so called" cerebral vasospasm after subarachnoid hemorrhage. Japan J Surg Cereb Stroke 2001;29(suppl):l–3
9. Ohta T, Baldwin M. Experimental mechanical arterial stimulation at the circle of Willis. J Neurosurg 1968;28:405–408
10. Echlin FA. Spasm of basilar and vertebral arteries caused by experimental subarachnoid hemorrhage. J Neurosurg 1965;23: 1–11
11. Ohta T, Kuroiwa T. Timing of CT scanning after SAH (letter). J Neurosurg 1985;63:817
12. Ohta T, Kuroiwa T, Sakaguchi I, Sakai N, Moriwaki K. Selective hypothermic perfusion of canine brain. Neurosurgery 1996;38: 1211–1215

26

Intravascular Adenoviral Gene Transfection of Monkey Cerebral Arteries Using Micro-Balloon Catheters

TOMOHITO HISHIKAWA, M.D., SHIGEKI ONO, M.D., MITSUHISA NISHIGUCHI, M.D., SHINSAKU NISHIO, M.D., KOJI TOKUNAGA, M.D., KENJI SUGIU, M.D., ISAO DATE, M.D.

Abstract

There are reports of gene transfection of the adventitial layers of cerebral arteries following intracisternal injection of various gene therapy vectors as well as some preliminary evidence for biological effects including antivasospastic efficacy Cisternal injections, however, have limitations, including the inability to achieve substantial gene transfection of the smooth muscle and endothelial cells. In addition, it is difficult to transfer vectors into specific anatomic locations. To overcome these problems, we examined the efficacy of intravascular transfection of cerebral arteries using adenovirus vectors and delivered by micro-balloon catheters in monkeys. Replication deficient adenovirus expressing β-galactosidase [Ad-Lac Z, 1.3×10^9 plaque-forming units (pfu)/mL] was prepared. A Commodore E micro-balloon catheter (inner diameter = 0.95 mm) was used to catheterize the intracranial left vertebral artery and to temporarily occlude it between two micro-balloon catheters filled with adenovirus. After saline irrigation, perfusion-fixation and 5-bromo-4-chloro-indolyl-p-D-galactopyranoside (X-gal) staining were performed 1 day after transfection. X-gal staining of the vertebral and basilar arteries showed a blue color segment of the left vertebral artery suggesting transfection with the β-galactosidase gene. This blue segment coincided precisely with the balloon-occluded area of the artery. Histochemical examination of frozen sections taken through the stained vertebral artery segments showed highly efficient transfection of the β-galactosidase gene into endothelial cells of the vertebral artery There were no X-gal positive cells in the media or adventitia. It is concluded that adenovirus was highly efficient in transfecting the cerebral endothelial cells. Micro-balloon catheters were valuable for local gene delivery to the cerebral arteries. More sophisticated systems to shorten the occlusion time of the cerebral arteries are needed.

There are reports of the use of gene therapy for cerebrovascular diseases.[1,2] Cerebral vasospasm after subarachnoid hemorrhage (SAH) has been a target of gene therapy, and moreover, some investigators reported the efficacy of cisternal injection of vectors as a delivery system.[3–5] Cisternal injections are relatively easy to perform, and cerebrospinal fluid circulation can carry vectors diffusely throughout the subarachnoid space, leading to widespread transfection of cells in the arachnoid and adventitia of the major subarachnoid

cerebral arteries. Cisternal injections, however, have some limitations. First, cisternal injection can generally only transfect exogenous genes into the adventitial layers of arteries. There is no or minimal gene transfection into the endothelial or smooth muscle cells. Second, it is difficult to transfect vectors into site-specific locations. Third, a large amount of vector is needed for transfection to the cerebral arteries, probably because the cerebrospinal fluid dilutes the vector. Endothelial cells have an important role in vascular biology, and endothelial dysfunction can cause or contribute to many cerebrovascular diseases, including cerebral vasospasm after SAH, atherosclerotic stenosis, and cerebral aneurysms. An endothelial-specific, highly efficient gene-transfection method would be a promising strategy for treatment of intracranial vascular lesions. In this report, we attempted to transfect adenoviral vectors encoding complimentary deoxyribonucleic acid for the β-galactosidase gene into intracranial arterial walls by an intravascular approach. We used micro-balloon catheters and examined the efficacy of transfection.

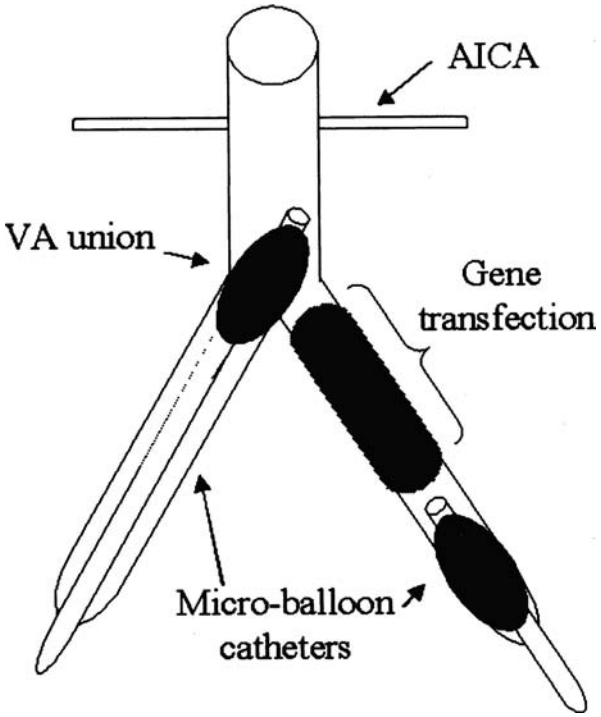


FIGURE 26–1 Schematic illustration of intra-arterial gene transfer. Micro-balloon catheters are inserted into the vertebral arteries bilaterally and β-galactosidase gene is transfected to the occluded lumen of the left vertebral artery. AICA, anterior inferior cerebellar artery; VA, vertebral artery.

Materials and Methods

Adenoviral Vectors

We used replication-deficient recombinant adenoviruses encoding the reporter gene, for *Escherichia coli* β-galactosidase driven by the immediate early cytomegalovirus (CMV) promoter. This replication-deficient adenoviral vector has deletions in the sequences of the E3, E1A, and E1B regions. These impair the ability of the virus to replicate. The CMV promoter was used to drive transcription of lac Z, the gene for β-galactosidase. Recombinant viruses were grown in human embryonic kidney (293) cells that provide the El early viral promoters and allow the virus to replicate. After purification, the virus was stored at -80°C until used.

Animals and Surgical Procedures

Four Japanese Macaca monkeys weighing 9 to 10 kg were used for this study. They were placed under general anesthesia with ketamine, 10 mg/kg intramuscularly, and sodium pentobarbital, 10 mg/kg intravenously. Size 6 French catheters were introduced as guiding catheters into each vertebral artery through the femoral arteries on each side. Subsequently, micro-balloon catheters (tip diameters = 0.95 mm, Commodore TM, Cordis Endovascular System, Miami, FL, USA) were inserted into each guiding catheter. The tip of the microcatheter on the left side was placed at the extra-intracranial junction of the left vertebral artery, and the right microcatheter was advanced to the vertebrobasilar junction. The balloons were inflated, and the isolated space between the balloons was irrigated with saline (5 mL, Fig. 26–1). The space was then filled with adenoviral vector [1.3 X 10^9 plaque-forming units (pfu)/mL, 5 mL volume] for 15 minutes. The balloons were deflated, and blood flow was restored. All the procedures and handling of animals were reviewed and approved by the standards of Okayama University Medical School.

X-gal Staining and Histological Examination

One day after adenovirus transfection, monkeys were euthanized with an overdose of sodium pentobarbital given by intravenous injection. Perfusion-fixation was performed, and the basilar and vertebral arteries were removed en bloc and placed in 2% paraformaldehyde overnight. The tissues were incubated in 30% sucrose solution for approximately 3 days and then incubated in 5-bromo-4-chloro-indolyl-p-D-galactopyranoside (X-gal) solution for 12 hours at 37°C. Frozen sections were cut from the tissue, placed on the slides, and counterstained with hematoxylin. Frozen sections were examined for positive staining of β-galactosidase (blue nuclei) by light microscopy.

Results

X-gal staining of the vertebrobasilar system macroscopically showed a blue colored segment of the left vertebral artery, consistent with β-galactosidase gene-transfection of the expected arterial segment. This blue segment was consistent with the area occluded in vivo by the balloon microcatheters. Light microscopy of frozen cross-sections of this vertebral artery segment with X-gal staining clearly demonstrated that the β-galactosidase gene was efficiently transfected into the endothelial cells. There were no X-gal positive cells in the media or adventitia (Fig. 26–2). On the other hand, no X-gal positive cells were found in any of the three layers of the right vertebral artery, which was not occluded (see Fig. 26–2).

Discussion

The adenoviruses (serotype 5) that we used as vectors in this study have 35 kilobasepair double strand genomic deoxyribonucleic acid and can efficiently transfect many types of cells in mammals. Transfection does not depend on cell replication, and cells in any stage of the cell cycle can be transfected. Thus, adenoviral vectors can be used to transfer genes to blood vessels despite the low rate of replication of cells in the vessel wall. Additionally, with regard to duration of expression of the transferred gene, adenoviral vectors are feasible for gene therapy of cerebral vasospasm after SAH because vasospasm does not develop for days after SAH and is itself only a transient phenomenon.

Several researchers reported that exogenous gene transfection by cisternal injection was efficacious against cerebral vasospasm after experimental SAH.[3–5] However, the transfected area was usually limited to the leptomeninges and the adventitia. It is difficult to transfect genes into the endothelium from the outside of the vessel wall due to the existence of various barriers in the arterial wall, including the internal elastic lamina. Since the vascular endothelium plays a major role in the regulation of biological processes controlling vascular tone, it may be more desirable to transfect exogenous genes directly into the endothelium for treatment of vessel diseases. Ooboshi et al reported that transgene expression with adenovirus was marked in the atherosclerotic endothelium, compared with normal endothelium in vitro.[6] They concluded that the atherosclerotic endothelium might be a good target for gene therapy. Taking these results into account, we consider that intracranial arterial stenosis may become a reasonable target for gene transfer, for instance, by adenovirus with stents from the inner surface of the vessels into the cerebral arterial stenosis.

Direct gene transfer into the endothelium of peripheral arteries, including the femoral and carotid arteries, has been reported.[7,8] We describe the first report to of transfection of an exogenous gene into the endothelium of cerebral arteries efficiently and selectively using micro-balloon catheters. Moreover, the transfected site was the exact location we targeted. Only a small amount of virus was needed compared with the case of cisternal injection.

Any proposal to use adenoviruses as vectors for gene therapy should take into account potential toxicity such as inflammation that may occur after exposure to adenoviruses.[2] An advantage of the present intravascular method for gene transfer is that tissues may suffer from less inflammation than after cisternal injection because blood flow carries excess virus away and a smaller amount of virus is needed overall. One major

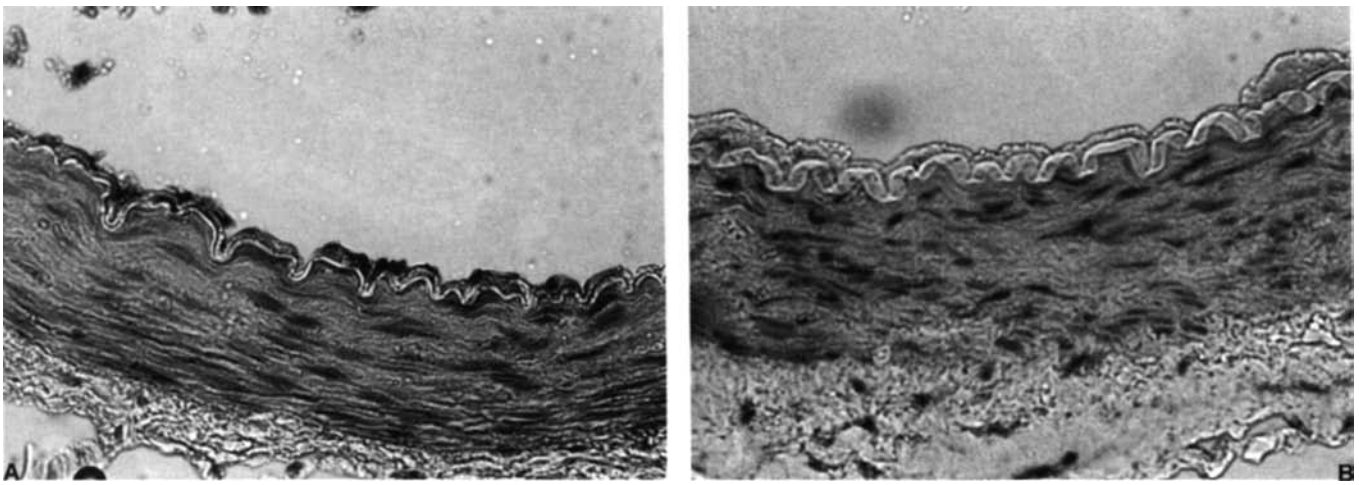


FIGURE 26–2 (A) Light microscopy of frozen sections of the left vertebral artery stained with X-gal demonstrates that the β-galactosidase gene expressed only in the endothelial cells. (B) The cross section of the control right vertebral artery does not show X-gal staining (original magnifications X 200).

disadvantage of the preceding system, however, would need to be overcome. This is the need to temporarily interrupt cerebral blood flow. In this study, temporary occlusion was for 15 minutes. Clearly temporary occlusion is associated with some risk of either or both cerebral ischemia and infarction. To surmount this problem, the development of new devices such as intravascular catheters or endothelium-specific vectors may be helpful. Interventional treatment for intracranial aneurysms, arteriovenous malformations, and intracranial arterial stenosis could become a more powerful tool if combined with stenting or coiling.

REFERENCES

1. Heistad DD, Faraci FM. Gene therapy for cerebral vascular disease. Stroke 1996;27:1688–1693

2. Weihl C, Macdonald RL, Stoodley M, Luders J, Lin G. Gene therapy for cerebrovascular disease. Neurosurgery 1999;44:239–253

3. Stoodley M, Weihl CC, Zhang Z, et al. Effect of adenovirus-mediated nitric oxide synthase gene transfer on vasospasm after experimental subarachnoid hemorrhage. Neurosurgery 2000;46:1193–1203

4. Toyoda K, Faraci FM, Watanabe Y, et al. Gene transfer of calcitonin gene-related peptide prevents vasoconstriction after subarachnoid hemorrhage. Circ Res 2000;87:818–824

5. Muhonen MG, Ooboshi H, Welsh MJ, Davidson BL, Heistad DD. Gene transfer to cerebral blood vessels after subarachnoid hemorrhage. Stroke 1997;28:822–829

6. Ooboshi H, Ibayashi S, Heistad DD, Fujishima M. Adenovirus-mediated gene transfer to cerebral circulation. Mech Ageing Dev 2000;116:95–101

7. Nabel EG, Plautz G, Nabel GJ. Site-specific gene expression in vivo by direct gene transfer into the arterial wall. Science 1990;249:1285–1288

8. Lemarchand P, Jones M, Yamada I, Crystal RG. In vivo gene transfer and expression in normal uninjured blood vessels using replication-deficient recombinant adenovirus vectors. Circ Res 1993;72:1132–1138

27

Oxyhemoglobin Potentiation of Thromboxane A_2-Induced Contraction of Porcine Basilar Arteries

ANTHONY JABRE, M.D., VIKRAM D. JADHAV, M.B.B.S.,
TONY JER-FU LEE, PH.D.

Abstract

Vasospasm and delayed cerebral ischemia remain a major cause of morbidity and mortality in the patient with aneurysmal subarachnoid hemorrhage (SAH). Thromboxane A_2 and oxyhemoglobin appear to play an important role in the cascade of events leading to the potent cerebral vasoconstriction that characterizes vasospasm. This study assessed the synergistic contractile effect of U-46619 (thromboxane A_2 analogue) and oxyhemoglobin in isolated porcine basilar arteries using the in vitro tissue bath technique. The results indicated that U-46619 caused constriction, whereas hemoglobin or oxyhemoglobin alone did not have a significant effect on basilar arteries with or without endothelial cells. Oxyhemoglobin, but not hemoglobin, potentiated U-46619-induced contraction. It is suggested that the functional synergism between thromboxane A_2 and oxyhemoglobin (but not hemoglobin) may play a role in pathogenesis of vasospasm following SAH.

Cerebral aneurysms are found in nearly 1% of the general population, with an estimated 36,000 individuals experiencing aneurysmal rupture and subarachnoid hemorrhage (SAH) each year in North America. Almost half of these patients die or suffer significant morbidity.[1] Advances in microsurgical techniques and anesthesia have reduced the hazards of surgery, allowed early aneurysm repair, and decreased the likelihood of rebleeding. Vasospasm following SAH, however, remains a leading cause of morbidity and mortality, with delayed cerebral ischemia developing in nearly 30% of patients. Vasospasm likely is the result of a cascade of complex actions triggered by the subarachnoid clot. Among the different substances contributing to the posthemorrhagic spasm are oxyhemoglobin and thromboxane A_2 (TxA_2).[2,3] Using the in vitro tissue bath technique, we studied the vasoconstrictive effects of hemoglobin, oxyhemoglobin, and TxA_2 agonist U-46619, in porcine basilar arteries.

Material and Methods

Adult porcine basilar arterial ring segments were dissected, cannulated, and mounted in tissue baths containing Krebs-bicarbonate solution (in mmol/L, NaCl 122.0; KCL 5.2; $CaCl_2$ 1.33; $MgSO_4$ 1.2; $NaCO_3$ 25.0; EDTA 0.03; L-ascorbic acid 0.01; and glucose 11.0, pH 7.4) equilibrated with a gas mixture of 95% O_2 and 5% CO_2 at 37°C.[4] Oxyhemoglobin was prepared from purified porcine hemoglobin (Sigma Chemicals, St. Louis, MO, USA) by reduction with sodium dithionite (1 mg dithionite/15 mg hemoglobin) and separated on a Sephadex G-25 column as previously described.[5] The concentration was determined using a BioRad protein assay. Drugs were

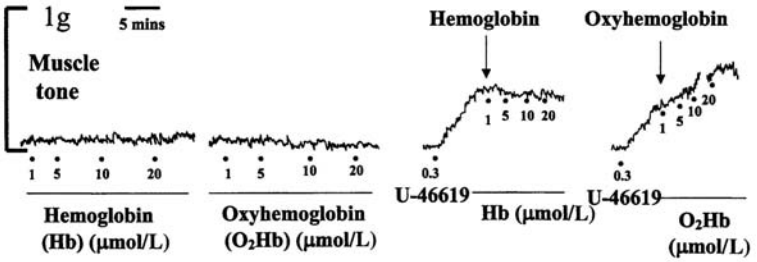


FIGURE 27–1 A representative tracing showing the vasomotor effect of hemoglobin (Hb, 1–20 μmol/L), oxyhemoglobin (O_2Hb, 1–20 μmol/L), and U-46619 (TxA_2 analogue, 0.3 μmol/L) on an endothelium-denuded porcine basilar arterial ring. Hemoglobin and oxyhemoglobin do not cause significant increases in tension alone, whereas oxyhemoglobin but not hemoglobin does augment tension in the presence of the TxA_2 agonist U-46619.

added directly to the tissue bath, and the concentrations of drugs reported were the final concentrations in the bath. The same tissue served as its own control in each ring preparation. Hemoglobin in incremental concentrations (1–20 μmol/L) was added in the tissue bath. After a thorough washout of the vessel segments with Krebs solution at 37°C, oxyhemoglobin in similar concentrations was applied. After rinsing, 0.3 μmol/L U-46619 (9, 11-dideoxy-9 α, 11 α-epoxymethanoprostaglandin F_2 α), a TxA_2 analogue (Sigma Chemical, St. Louis, MO, USA), was added to the tissue bath. When steady active tone was reached, a similar concentration of either hemoglobin or oxyhemoglobin was added. The magnitude of vasoconstrictor responses was expressed as a percent of the constriction induced by KCl (80 mmol/L), which was added at the end of each experiment. The data were computed as means ± standard error of the mean (SEM) and statistically evaluated by analysis of variance (ANOVA) for repeated measures and multigroup comparisons.

Results

Hemoglobin and oxyhemoglobin in increasing concentrations (1–20 μmol/L) did not cause significant constriction of porcine basilar arteries with or without endothelial cells. In the presence of active muscle tone induced by U-46619 (0.3 (μmol/L), oxyhemoglobin but not hemoglobin further raised contractions (Figs. 27–1 and 27–2) in a concentration-dependent manner in both endothelium-present and denuded arterial segments.

Discussion

Hemoglobin has been shown to trap nitric oxide (NO) released from the perivascular neurons and the endothelial cells.[6] Although vasospasm following SAH is due to multifactorial events, its exact pathogenesis is yet to be determined. Zubkov et al found that mitogen-activated protein kinase, a tyrosine kinase substrate, was activated by oxyhemoglobin, causing vasoconstriction.[3] Contraction induced by oxyhemoglobin may also be due to its oxidation into methemoglobin, producing free radicals and initiating the arachidonic acid cascade and lipid peroxidation.[7] The higher the concentration of oxyhemoglobin in the bloody cerebrospinal fluid, the more potent is the constriction.[8] TxA_2, which is endogenously present in platelets, endothelial cells, and arterial smooth muscle

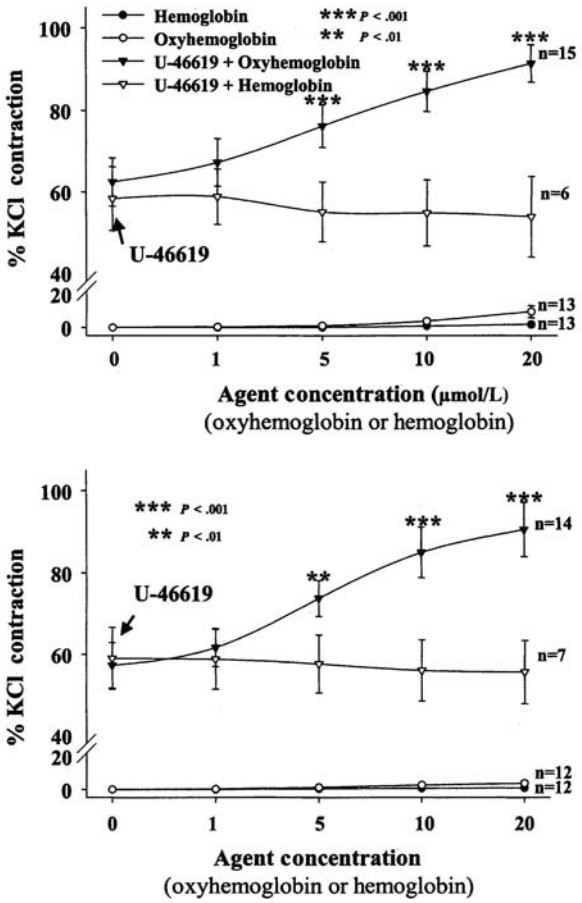


FIGURE 27–2 Graphs of quantitative analysis of effects of hemoglobin (1–20 μmol/L) and oxyhemoglobin (1–20 μmol/L) alone and in the presence of U-46619 (0.3 μmol/L) in rings of porcine basilar artery (A) with or (B) without endothelium. The contraction was estimated as a percent of contraction in response to KCl, 80 mmol/L. Values are mean ± SEM; *n* indicates number of experiments (*** $p < .001$, ** $p < .01$).

cells, is significantly increased in the cerebrospinal fluid after SAH.[9] It is a powerful vasoconstrictor and stimulates platelet aggregation. The vasoconstriction induced by TxA_2 is due to enhanced Ca^{2+} influx and the release of Ca^{2+} from the internal stores.[10] Synergism among endogenous vasoconstrictors has been previously described. Hempelmann et al noted a synergistic effect between U-46619 and 5-hydroxtryptamine causing an increased vasoconstriction.[2] Our present findings showed such a synergistic mechanism between two of the major putative spasmogens in cerebral vasospasm after SAH; namely, TxA_2 and oxyhemoglobin. Oxyhemoglobin has been suggested to cause vasospasm by producing an increase in the intracellular levels of inositol-triphosphate, which promotes a rise in the intracellular calcium.[11] Activation of phospholipase C has been suggested to be a critical step in the development of vasospasm, and the sustained contraction seen in cerebral vasospasm may arise from phospholipase C-dependent factors such as protein kinase C.[11,12]

Conclusion

The present findings suggest that the synergism between TxA_2 and oxyhemoglobin may contribute to the complex cascade of events leading to delayed cerebral vasospasm following SAH.

REFERENCES

1. Ljunggren B, Saveland H, Brandt L, Zygmunt S. Early operation and overall outcome in aneurysmal subarachnoid hemorrhage. J Neurosurg 1985;62:547–551
2. Hempelmann RG, Pradel RH, Barth HL, Mehdorn HM, Ziegler A. Interactions between vasoconstrictors in isolated human cerebral arteries. Acta Neurochir (Wien) 1997;139:574–581
3. Zubkov AY, Rollins S, McGehee B, Parent A, Zhang JH. Relaxant effect of U0126 in hemolysate-, oxyhemoglobin-, and bloody cerebrospinal fluid-induced contraction in rabbit basilar artery. Stroke 2001;32:154–161
4. Si ML, Lee TJ. Alpha7-nicotinic acetylcholine receptors on cerebral perivascular sympathetic nerves mediate choline-induced nitrergic neurogenic vasodilation. Circ Res 2002;91:62–69
5. Linnik MD, Lee TJF. Effect of hemoglobin on neurogenic responses and cholinergic parameters in porcine cerebral arteries. J Cereb Blood Flow Metab 1989;9:219–225
6. Lee TJF. Nitric oxide and the cerebral vascular function. J Biomed Sci 2000;7:16–26
7. Sano K, Asano T, Tanishima T, Sasaki T. Lipid peroxidation as a cause of cerebral vasospasm. Neurol Res 1980;2:253–272
8. Kanamaru K, Waga S, Kojima T, Fujimoto K, Niwa S. Endothelium-dependent relaxation of canine basilar arteries, II: Inhibition by hemoglobin and cerebrospinal fluid from patients with aneurysmal subarachnoid hemorrhage. Stroke 1987;18:938–943
9. Parfenova H, Shibata M, Leffler CW. Subarachnoid blood causes pial arteriolar constriction in newborn pigs. Stroke 1993;24:1729–1734
10. Wendling WW, Harakal C. Effects of prostaglandin F2 alpha and thromboxane A2 analogue on bovine cerebral arterial tone and calcium fluxes. Stroke 1991;22:66–72
11. Vollrath BA, Weir BK, Macdonald RL, Cook DA. Intracellular mechanisms involved in the responses of cerebrovascular smooth muscle cells to hemoglobin. J Neurosurg 1994;80:261–268
12. Laher I, Zhang JH. Protein kinase C and cerebral vasospasm. J Cereb Blood Flow Metab 2001;21:887–906

28

Cytosolic Calcium Oscillations Induced by Cisternal Cerebrospinal Fluid from SAH Patients

WOLFRAM SCHARBRODT, M.D., CLAUDIA SCHÄFER, M.D., DIETER-KARSTEN BÖKER, M.D., MICHAEL H. PIPER, M.D., WOLFGANG DEINSBERGER, M.D.

Abstract

After a subarachnoid hemorrhage (SAH), cerebral endothelial cells are exposed to extravascular hemoglobin as well as products of reactions between the surrounding tissue and the immune system. The present study investigated whether these factors are able to influence the endothelial cytosolic calcium concentration ($[Ca^{2+}]$) after SAH. As an experimental model, human umbilical cord vein endothelial cells (HUVEC) were incubated with cisternal cerebrospinal fluid (CSF) from eight patients with SAH. Four of these patients had cerebral vasospasm. Native CSF was used in control experiments. HUVEC were loaded with the fluorescence Ca^{2+} indicator Fura-2. The emitted fluorescence light was collected with an inverted microscope attached to a video imaging system. Incubation of HUVEC with SAH-CSF provoked cytosolic Ca^{2+} oscillations in six of eight cases. After perfusion with CSF sampled from patients with cerebral vasospasm (n = 4), endothelial Ca^{2+} oscillations appeared immediately in all cases. The oscillation frequency was 0.27 ± 0.02/min (mean value ± standard error of the mean [SEM]; n = 44 cells). In the presence of thapsigargin (50 nmol/L), an inhibitor of endoplasmic reticulum Ca^{2+} adenosine triphosphatase (ATPase), the oscillations ceased in all cases. In control experiments with native CSF no oscillations were observed in six cases; in two cases a minor number of cells showed transient Ca^{2+} peaks with a frequency of 0.04 ± 0.03/min (n = 8 cells). This study shows that cisternal SAH-CSF, especially from patients with cerebral vasospasm, is able to induce endothelial cell cytosolic Ca^{2+} oscillations. These oscillations can be blocked by thapsigargin. This indicates that the oscillations are dependent on the function of endoplasmic reticulum Ca^{2+} ATPase.

Vascular endothelial cells act as an interface between blood and the vascular wall, actively regulating permeability, immune defenses, coagulation, and vascular tone. Due to their position, the endothelial cells are influenced by mechanical and chemical stimuli of blood and the surrounding tissue. The intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) is also linked to these stimuli.[1] Most functions of vascular endothelial cells depend on $[Ca^{2+}]_i$, including permeability and synthesis of nitric oxide and, as a result, vascular tone.[2] Inflammation may

be linked to $[Ca^{2+}]_i$ because alterations in $[Ca^{2+}]_i$ may increase nuclear factor-kappa B (NF-KB) transcription.[34]

After a subarachnoid hemorrhage (SAH), cerebral endothelial cells are exposed to extravascular hemoglobin as well as products of reactions between the surrounding tissue and the immune system. This study investigated whether these factors are able to influence the endothelial cytosolic Ca^{2+} concentration after SAH.[5]

Methods

As an experimental model, human umbilical cord vein endothelial cells (HUVEC) were loaded with the fluorescence Ca^{2+} indicator Fura-2/AM. The excitation wavelengths were 340 nm and 380 nm. The emitted fluorescence light was collected using an inverted microscope attached to a video imaging system and analysis computer. Regions of interest were marked enveloping the single cells, and the $[Ca^{2+}]_i$ was determined by calculating the 340 nm:380 nm ratio in respect to the background signal. At the beginning of the measurement the cells were incubated with buffer solution for 10 minutes. The solution then was replaced with cerebrospinal fluid (CSF). In further experiments 30 minutes later 50 nmol/L thapsigargin was added to the CSF.[6] The endothelial cells were incubated with cisternal CSF from eight patients with SAH. The SAH was classified as Fisher grade 3 or 4.[7] The CSF was sampled on the fifth or sixth day after bleeding. Four of these patients had cerebral vasospasm. The vasospasm in all cases was detected by transcranial Doppler ultrasound, and in two cases it was confirmed by cerebral angiography. In control experiments we used native CSF obtained during myelography

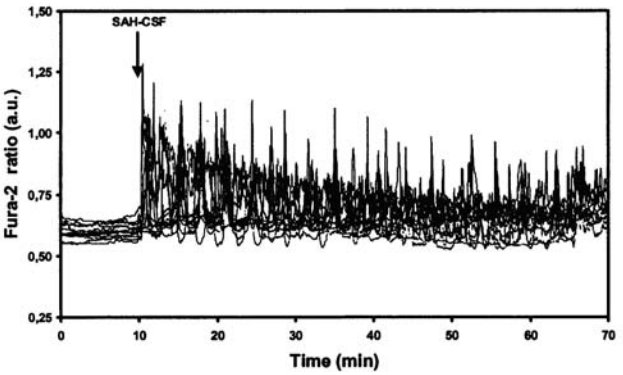


FIGURE 28–1 Tracings of the 340 nm:380 nm ratio that represents the cytosolic Ca^{2+} concentration of a single cell. After an initial incubation for 10 minutes, the buffer solution is replaced by cisternal cerebrospinal fluid (CSF) from a patient with cerebral vasospasm (subarachnoid hemorrhage [SAH]-CSF). Oscillations in cytosolic Ca^{2+} develop.

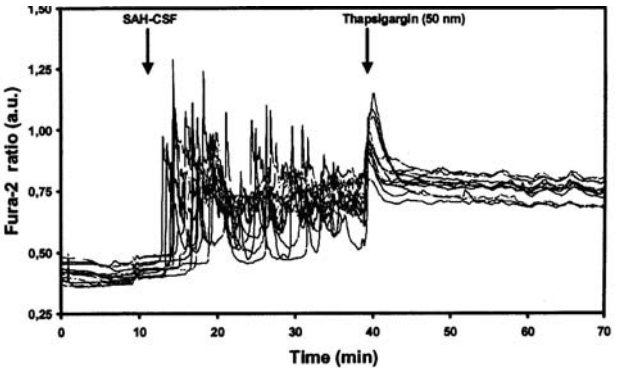


FIGURE 28–2 Tracings of the 340 nm:380 nm ratio, representing cytosolic Ca^{2+} concentration. After 12 minutes, Ca^{2+} oscillations develop after addition of cerebrospinal fluid (CSF) from a patient with vasospasm (subarachnoid hemorrhage [SAH]-CSF). At ~40 minutes, thapsigargin (50 nmol/L), an inhibitor of the endoplasmic reticulum Ca^{2+}-adenosine triphosphatase, was added, leading to loss of Ca^{2+} oscillations.

Results

Incubation of HUVEC with SAH-CSF provoked cytosolic Ca^{2+} oscillations in six of eight cases. After perfusion with CSF sampled from patients with cerebral vasospasm (n = 4), endothelial Ca^{2+} oscillations appeared immediately in all cases (Fig. 28–1). The oscillation frequency was 0.27 ± 0.02/min (mean value ± SEM; n = 44 cells). After addition of thapsigargin (50 nmol/L), an inhibitor of endoplasmic reticulum Ca^{2+-}-adenosine triphosphatase (ATPase), the oscillations ceased in all cases (n = 6, Fig. 28–2). In control experiments with native CSF no oscillations were observed in six cases. In two cases a minor number of cells showed transient Ca^{2+} peaks with a frequency of 0.04 ± 0.03/min (n = 8 cells, Fig. 28–3).

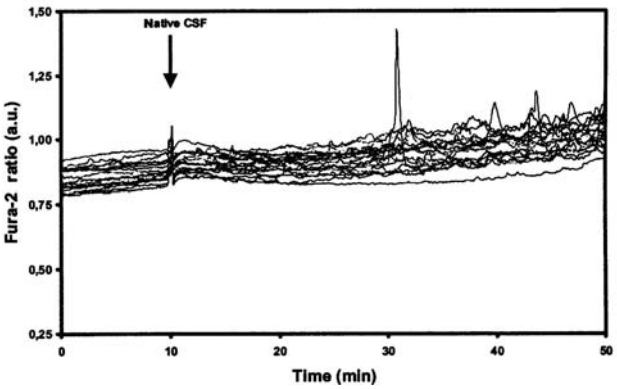


FIGURE 28–3 Tracings of the 340 nm:380 nm ratio that represents the cytosolic Ca^{2+} concentration of a single cell. After an initial incubation for 10 minutes, the buffer solution is replaced by native, control cerebrospinal fluid (CSF). Only infrequent oscillations in cytosolic Ca^{2+} develop.

Discussion

Cisternal CSF obtained from patients with SAH, especially from patients with cerebral vasospasm, was shown to induce endothelial cell cytosolic Ca^{2+} oscillations with a frequency of 0.27 ± 0.02/min. These oscillations were blocked by thapsigargin. This indicates that the oscillations are dependent on the function of endoplasmic reticulum Ca^{2+-}-ATPase.[5] The cytosolic Ca^{2+} rise when thapsigargin was added is induced by the depletion of internal Ca^{2+} stores. The endothelial cytosolic calcium oscillations may contribute to an increased NF-KB transcriptional activity and thus to expression vascular cell adhesion molecule 1.[8] This leads possibly to local inflammation that could contribute to cerebral vasospasm.

REFERENCES

1. Faraci FM, Heistad DD. Regulation of the cerebral circulation: role of endothelium and potassium channels. Physiol Rev 1998;78:53–97
2. Tran QK, Ohashi K, Watanabe H. Calcium signalling in endothelial cells. Cardiovasc Res 2000;48:13–22
3. Dolmetsch RE, Xu K, Lewis RS. Calcium oscillations increase the efficiency and specificity of gene expression. Nature 1998;392:933–936
4. Visgrady A, Lakos Z, Czimbalek L, Somogyi B. Stimulus-dependent control of inositol 1,4,5-trisphosphate-induced Ca^{2+} oscillation frequency by the endoplasmic reticulum Ca^{2+}-ATPase. Biophys J 2001;81:1398–1405
5. Zhang H, Weir BKA, Macdonald RL, et al. Mechanisms of $[Ca^{2+}]_i$ elevation induced by erythrocyte components in endothelial cells. J Pharmacol Exp Ther 1996;277:1501–1509
6. Schafer M, Bahde D, Bosche B, et al. Modulation of early $[Ca^{2+}]_i$ rise in metabolically inhibited endothelial cells by xestospongin C. Am J Physiol Heart Circ Physiol 2001;280:1002–1010
7. Fisher CM, Kistler JP, Davis JM. Relation of cerebral vasospasm to subarachnoid hemorrhage visualized by computerized tomographic scanning. Neurosurgery 1980;6:1–9
8. Quinlan KL, Naik SM, Cannon G, et al. Substance P activates coincident NF-AT and NF-KB-dependent adhesion molecule gene expression in microvascular endothelial cells through intracellular calcium mobilization. J Immunol 1999;163:5656–5665

29

Nicotine Exposure Potentiates Vasoconstriction of Canine Basilar Artery via Protein Kinase C Activation and Attenuation of Nitric Oxide Synthesis

MASAYO KOIDE, M.S., SHIGERU NISHIZAWA, M.D., PH.D., SEIJI YAMAMOTO, M.D., PH.D., MITSUO YAMAGUCHI, M.D., YUICHIRO NONAKA, M.D., SUSUMU TERAKAWA, M.D., PH.D.

Abstract

Cigarette smoking is an important risk factor for cerebrovascular disorders. Among many compounds in cigarette smoke, nicotine has been considered to most significantly affect cerebral vascular tone. However, the precise pharmacological effects of nicotine on cerebral arterial tone have not been clarified. This study investigated the pharmacological mechanism of action of nicotine on the regulation of cerebral arterial tone and the effect of nicotine on cerebral vasospasm after subarachnoid hemorrhage (SAH). The effect of nicotine was assessed on canine basilar artery contracted with uridine 5'-triphosphate (UTP) and relaxed by endothelium-dependent vasodilatation in an isometric tension study. Synthesis of endogenous nitric oxide (NO) was measured in cultured vascular endothelial cells using the fluorescent indicator, diaminofluorescein-FM diacetate. Protein kinase C (PKC) activation was assessed in canine basilar arteries by enzyme immunoassay Nicotine significantly enhanced UTP-induced contraction, attenuated endothelium-dependent vasodilatation by substance-P, decreased NO synthesis in cultured vascular endothelial cells, and increased PKC activity in the canine basilar artery De-endothelialization itself increased PKC activity in the vascular smooth muscle. Nicotine significantly enhanced PKC activity to a greater extent than de-endothelialization. The increase in PKC activity in response to nicotine was not statistically significantly different between arteries with and without endothelium. These results indicate that pharmacological action of nicotine is to attenuate endothelial function, resulting in decreased NO synthesis. Nicotine also caused direct and indirect activation of PKC in vascular smooth muscle. These results suggest that nicotine could induce severe cerebral vasoconstriction after SAH.

Cigarette smoking is a significant risk factor for cerebrovascular disorders, including cerebral vasospasm after subarachnoid hemorrhage (SAH).[1,2] Among the thousands of compounds in cigarette smoke, nicotine has been reported to have the most significant effect on various tissues. With regard to cerebral arteries, topical application of nicotine causes vasodilation by releasing nitric oxide (NO) from perivascular nerves.[3]

However, little is known about the effects of chronic exposure of tissues such as cerebral arteries to low concentrations of nicotine that might occur with chronic cigarette smoking. To clarify the effects of long-term exposure to low concentrations of nicotine on the properties of cerebral artery, arterial contractility, endothelium-dependent vasodilatation, endothelial function, and protein kinase C (PKC) activity were examined. The long-term exposure to low concentrations was designed to mimic smoking.

Methods

A concentration of 10–^{6} mol/L nicotine was used based on the serum concentration of nicotine in habitual smokers.[4] Arterial contractility was assessed in an isometric tension study using canine basilar arteries. The magnitude of uridine 5'-triphosphate (UTP)-induced contraction before and after incubation with nicotine were compared in the same artery. Endothelium-dependent vasodilatation also was measured in the same arteries under isometric tension. Isolated canine basilar artery was incubated with or without nicotine for 1 hour. After the artery was precontracted with UTP (10^{-5} mol/L), substance P, an endothelium-dependent vasodilator, was applied cumulatively At the end of the experiment, papaverine (10^{-4} mol/L) was administered to obtain the maximum relaxation, and the magnitude of substance P-induced vasodilatation was expressed as a percent of the maximum relaxation to papaverine, which was set at 100%. The synthesis of NO, an endothelium-derived relaxing factor, was analyzed as a marker of endothelial function. Intracellular NO synthesis was directly detected in cultured vascular endothelial cells using diaminofluorescein-FM diacetate (DAF-FM/DA), a fluorescent NO indicator. The cultured endothelial cells were treated with nicotine for 24 hours before the measurement of fluorescent intensity, after which time they were stimulated with substance P (10^{-6} mol/L), which was the same concentration as that used in the experiments already described testing endothelium-dependent vasodilatation. The fluorescent intensity was expressed as a percent of that before stimulation of the same cells.

PKC activation is known to involve translocation from the cytosolic to the membrane fraction. PKC activity was therefore measured in the membrane fraction of canine basilar arteries using an enzyme-linked immunosorbent assay (ELISA). The PKC activities were compared between arteries with and without nicotine exposure and between intact and endothelium-denuded arteries. For statistical analysis, p values < .05 were considered to be significant.

Results

In the isometric tension study, UTP-induced vasoconstriction was significantly potentiated by chronic exposure to nicotine (Fig. 29–1A). Substance P induced dose-dependent vasodilatation of canine basilar artery. In nicotine-treated arterial rings, endothelium-dependent vasodilatation in response to substance P was significantly attenuated (Fig. 29–1B).

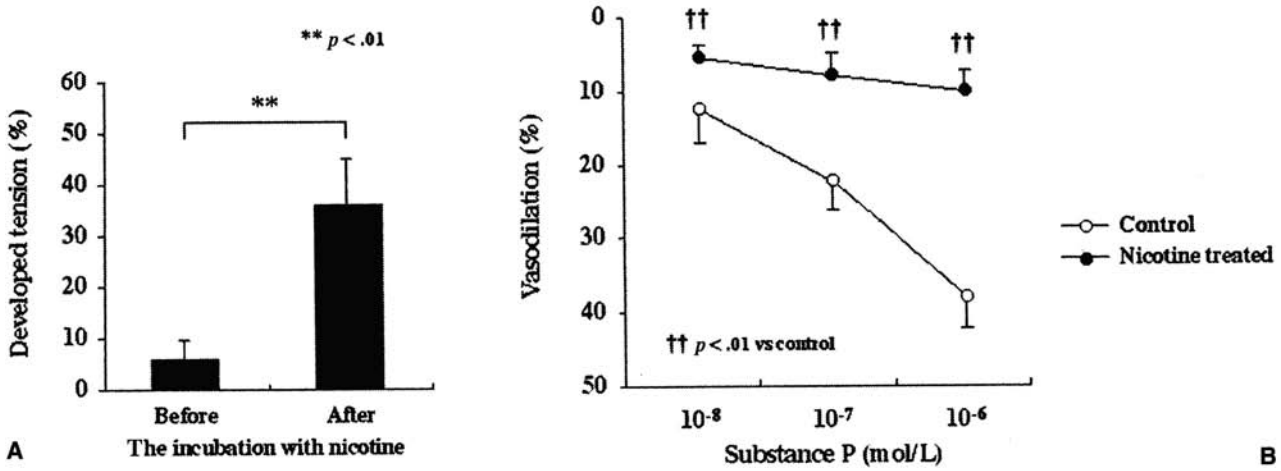


FIGURE 29–1 Effects of chronic exposure of canine basilar artery suspended under isometric tension to a low concentration of nicotine (10^{-6} mol/L). (A) Effects were examined on uridine-5'-triphosphate (UTP, 10^{-5} mol/L)-induced contraction and (B) on substance P-induced vasodilatation. In (A) summarized data of UTP-induced tension before and after nicotine exposure show a significant potentiation of contraction after exposure to nicotine. In (B) dose-response curves show the effect of nicotine exposure on substance P-induced vasodilatation. The artery was incubated without (open circles) or with (closed circles) nicotine (10^{-6} mol/L). All data are expressed as means ± standard error of the mean (SEM) (n = 4 each).

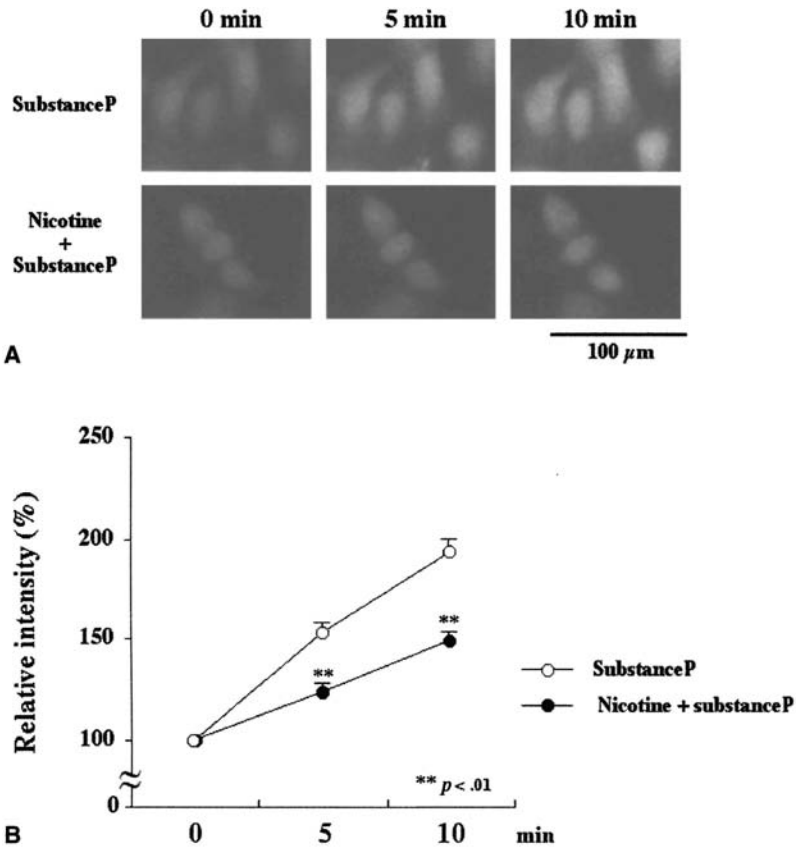


FIGURE 29–2 Effect of chronic exposure of cultured vascular endothelial cells to a low concentration of nicotine. The end point was substance P-induced nitric oxide (NO) synthesis. (A) Fluorescent images showing the intracellular synthesis of NO in cultured endothelial cells. (B) Summarized data of the fluorescent intensity showing significant inhibition in the synthesis of NO 5 and 10 minutes after exposure.

The synthesis of NO in cultured vascular endothelial cells was significantly diminished by chronic incubation with nicotine (Fig. 29–2). Chronic exposure to nicotine produced a dose-dependent enhancement of PKC activity, and de-endothelialization itself increased PKC activity (Fig. 29–3). Moreover, chronic exposure to nicotine enhanced PKC activity in endothelium-denuded arteries more than de-endothelialization (see Fig. 29–3). The difference in PKC activity in nicotine-treated arteries with and without endothelium was not significantly different (see Fig. 29–3).

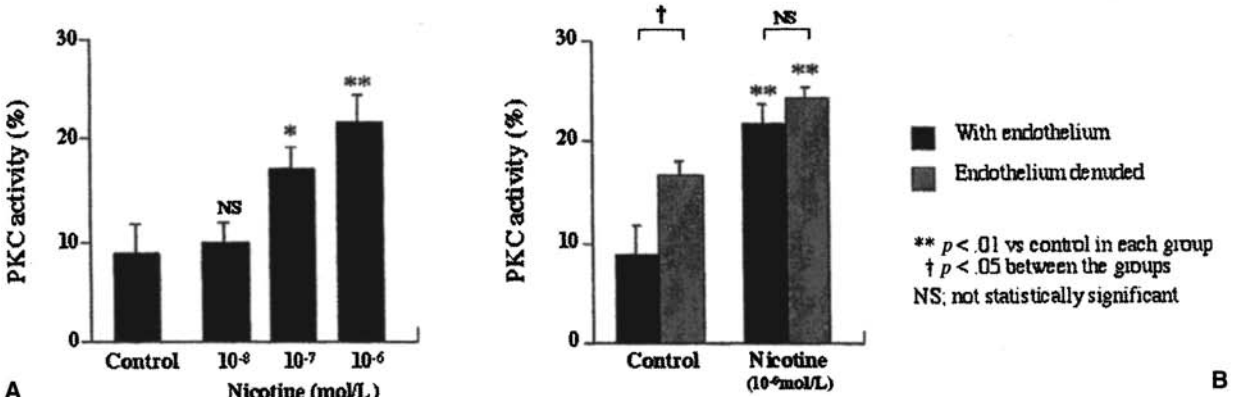


FIGURE 29–3 Effect of chronic exposure to a low concentration of nicotine on protein kinase C (PKC) activity in canine basilar arteries. Data are expressed as means ± SEM. (A) Dose-dependent enhancement of PKC activity by nicotine in canine basilar artery with endothelium. (B) PKC activity in canine basilar arteries with (closed columns) or without endothelium (shaded columns) and with or without exposure to nicotine. Data are means ± SEM (n = 4 per group). There is significant enhancement in PKC activity after endothelial removal and exposure to nicotine.

Discussion

This study reports that chronic exposure to nicotine, in a manner designed to mimic chronic cigarette smoking, caused potentiation of UTP-induced vasoconstriction and attenuation of substance P-induced vasodilatation in the dog basilar artery suspended under isometric tension. The attenuation of endothelium-dependent vasodilatation suggests that chronic exposure to nicotine causes endothelial dysfunction. The study measuring the synthesis of NO using DAF-FM/DA in cultured endothelial cells directly shows endothelial dysfunction in response to nicotine exposure and supports the data obtained in the isometric tension study. Chronic exposure to nicotine enhanced PKC activity in canine basilar artery in a dose-dependent fashion. Removal of the endothelium itself increased PKC activity, however, suggesting that PKC activity in the intact artery might be controlled in part by endothelial function. In other words, once endothelial dysfunction occurs, PKC in the artery would be activated. PKC activity in the intact artery and in the endothelium-denuded artery was further enhanced by nicotine exposure than by simple endothelial removal alone. These results indicate that the mechanism of PKC activation in response to chronic exposure to nicotine might be caused not only by the disinhibition of endothelium on the PKC activation mechanism but also by a direct activation effect of nicotine on PKC.

Conclusion

Chronic exposure to low concentrations of nicotine caused potentiation of UTP-induced vasoconstriction and attenuation of substance P-induced vasodilatation in cerebral arteries and reduction of NO synthesis in cultured vascular endothelial cells. There was evidence for a direct and indirect enhancement of PKC activity. These results indicate that chronic exposure to nicotine may induce cerebral vasoconstriction in response to other agents. Smoking might cause not only potentiation of contraction but also PKC activation. PKC activation plays a role in cerebral vasospasm after SAH.[5–8] The cerebral arteries exposed to nicotine might be prone to contract compared with intact arteries, suggesting that smokers might carry the risk of severe cerebral vasospasm after SAH.

REFERENCES

1. Khaw KT, Barrett-Connor E, Suarez L, Criqui MH. Predictors of stroke-associated mortality in the elderly. Stroke 1984;15:244–248
2. Petitti DB, Wingerd J, Pellegrin F, Ramcharan S. Risk of vascular disease in women: smoking, oral contraceptives, noncontraceptive estrogens, and other factors. JAMA 1979;242:1150–1154
3. Toda N, Uchimura M, Okamura T. Prejunctional modulation of nitroxidergic nerve function in canine cerebral arteries. Brain Res 1995;700:213–218
4. Armitage AK, Dollery CT, George CF, Houseman TH, Lewis PJ, Turner DM. Absorption and metabolism of nicotine from cigarettes. BMJ 1975;4:313–316
5. Nishizawa S, Nezu N, Umemura K. Direct evidence for a key role of protein kinase C in the development of vasospasm after subarachnoid hemorrhage. J Neurosurg 1992;76:635–659
6. Nishizawa S, Yamamoto S, Yokoyama T, Ryu H, Umemura K. Chronological changes of arterial diameter, cGMP, and protein kinase C in the development of vasospasm. Stroke 1995;26:1916–1921
7. Nishizawa S, Obara K, Nakayama K, et al. Protein kinase C and a are involved in the development of vasospasm after subarachnoid hemorrhage. Eur J Pharmacol 2000;398:113–119
8. Koide M, Nishizawa S, Ohta S, Yokoyama T, Namba H. Chronological changes of the contractile mechanism in prolonged vasospasm after subarachnoid hemorrhage: from protein kinase C to protein tyrosine kinase. Neurosurgery 2002;51:1468–1476

30

Experimental SAM Upregulates 5-HT$_{1B}$ Receptors in Rat Cerebral Arteries

JACOB HANSEN-SCHWARTZ, M.D., PH.D., NATALIE LØVLAND HOEL, B.S., CANG-BAO XU, M.D., PH.D., NIELS- AAGE SVENDGAARD, M.D., PH.D., LARS EDVINSSON, M.D., PH.D.

Abstract

Cerebral vasospasm after subarachnoid hemorrhage leads to reduced blood flow in the brain. Organ culture has been shown to induce changes in the receptor phenotype of cerebral arteries. This study investigated whether experimental SAH might be associated with changes in the serotonin (5-hydroxytryptamine or 5-HT) receptor phenotype. Experimental subarachnoid hemorrhage (SAH) was induced in rats by injection of autologous arterial blood into the prechiasmatic cistern. Two days later the middle cerebral, posterior communicating, and basilar arteries were harvested and examined functionally using a sensitive in vitro pharmacological method, and molecularly using quantitative real-time reverse transcription-polymerase chain reaction (RT-PCR). In the middle cerebral and basilar arteries the 5-HT$_{1B}$ receptor was upregulated based on functional and molecular analysis. In response to selective 5-HT$_1$ receptor agonists, the pEC$_{50}$ was increased (one log unit in the middle cerebral and half a unit in the basilar artery) and the potency similarly doubled in the middle cerebral and increased by 50% in the basilar artery In addition, an approximately fourfold increase in the number of copies of messenger ribonucleic acid coding for the 5-HT$_{1B}$ receptor was demonstrated by quantitative real-time RT-PCR. In the posterior communicating artery no upregulation of the 5-HT$_{1B}$ receptor was observed. Changes in receptor phenotype in favor of contractile receptors provide a challenging new perspective in the sequence of events leading from SAH to the actual development of cerebral vasospasm.

For many years 5-hydroxytryptamine (5-HT, serotonin) was thought of as a key player in the pathogenesis of cerebral vasospasm because of the powerful contractile response generated by 5-HT. Sources of 5-HT include platelets, the serotonergic innervation of cerebral vessels extending from the raphe nuclei,[1] and the recently discovered innervation of the basilar artery by serotonergic chemosensitive neurons.[2] The interest in 5-HT as a key player has in the recent decades lost impetus mainly due to the lack of clinical efficacy of 5-HT antagonists in treating vasospasm.[3] However, the lack of clinical effect does not per se exclude the involvement of 5-HT, but does perhaps point to a multifactorial pathogenesis. Organ culture of whole segments of the basilar artery from rats induces upregulation of contractile receptors, including the contractile

5-HT$_{1B}$ receptor. This upregulation renders the arterial segment considerably more sensitive to 5-HT.[4] Because this intrinsic ability of an artery to upregulate contractile receptors may be of pathophysiological significance, we set out to investigate possible upregulation of this receptor after induction of experimental subarachnoid hemorrhage (SAH) in rats.

Methods

Induction of SAH, pharmacological examination in vitro, and measurement of receptor messenger ribonucleic acid (mRNA) were done according to the methods described elsewhere in this monograph.[5] For the construction of concentration-contraction curves, 5-carboxamidotryptamine (5-CT, a specific 5-HT$_1$ receptor agonist) in concentrations from 10^{-10} to $10^{-4.5}$ mol/L was used. The specific 5-HT$_{1B}$ antagonist GR55562 was employed at a concentration of 10^{-6} mol/L to characterize responses. Primer sequences used in the quantitative measurement of receptor mRNA have been published.[6]

Results

In Vitro Pharmacology

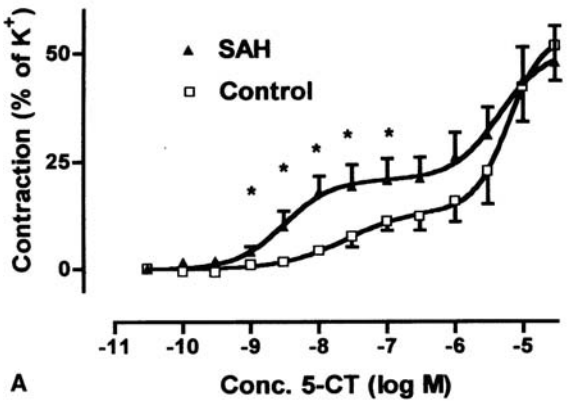
The biphasic in vitro response to 5-CT was clearly enhanced in the middle cerebral and basilar arteries of rats with SAH (Fig. 30–1). GR55562 shifted the first phase to the right and removed the 5-HT$_{1B}$ component of the second phase, lowering the E_{max} observed at the available maximal concentration, but not affecting the pEC_{50} of the second phase (data not shown). This indicates an upregulated 5-HT$_{1B}$ response. In the middle cerebral artery, the pEC_{50} of the 5-HT$_{1B}$ phase was increased from 7.6 ± 0.2 (mean ± standard error of the mean [SEM]) to 8.5 ± 0.1, and the E_{max} of the first phase was increased from 14 ± 3% to 21 ± 5%. There was no significant difference between the second 5-HT$_{2A}$ phase of the contraction for which pEC_{50} was 5.3 ± 0.2 and 5.2 ± 0.1 and E_{max} 48 ± 8% and 51 ± 8%, respectively. In the basilar artery, a similar upregulation was noted. In SAH rats, pEC_{50} of the 5-HT$_{1B}$ phase was 8.2 ± 0.2 compared with 7.7 ± 0.1 in control rats, and E_{max} was 27 ± 7% in SAH rats and 11 ± 3% in control rats. As in the middle cerebral artery, the second 5-HT$_{2A}$ phase of the contraction was similar in SAH and control rats (pEC_{50} 5.5 ± 0.2 and 5.1 ± 0.1 and E_{max} 62 ± 9% and 59 ± 8%, respectively for SAH and control). In the posterior communicating artery, a part of the circle of Willis, SAH did not cause a significant change in the 5-HT$_{1B}$ phase. The concentration-contraction curve for the posterior communicating artery from SAH rats was not affected by the addition of GR55562, indicating that the contraction induced was not dependent on the 5-HT$_{1B}$ receptor.

Quantitative Real-Time PCR

The functional data described above were substantiated by measurements of the number of copies of 5-HT$_{1B}$ receptor mRNA in the arteries. In the middle cerebral and basilar arteries, the number of copies of mRNA coding for 5-HT$_{1B}$ receptor were 3.8 ± 0.8 and 3.9 ± 1.2 times higher, respectively, in the SAH rats compared with the control rats (Fig. 30–2). In the posterior communicating artery, there was no significant difference between SAH rats and control rats. The mRNA for the 5-HT$_{1D}$ receptor could not be detected in significant amounts (data not shown).

Conclusion and Discussion

Combining the observations done using endothelin[5] with those obtained with 5-HT in the present study, it has been shown that blood injected in the subarachnoid space of the rat clearly leads to altered pharmacological



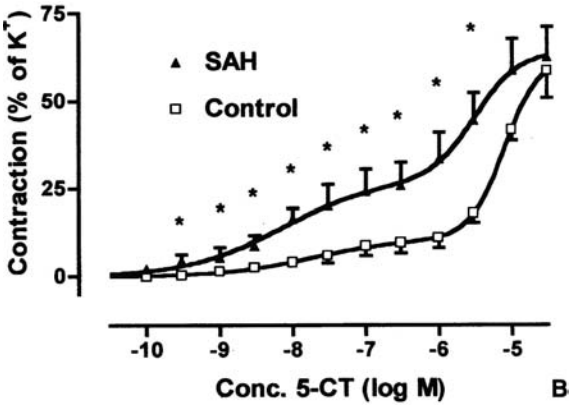


FIGURE 30–1 Graphs of concentration-contraction curves showing (A) the effect of 5-carboxamidotryptamine (5-CT) on the middle cerebral and (B) basilar arteries, respectively. In both arteries an enhanced 5-CT response is observed in rats with experimental subarachnoid hemorrhage compared with sham operated rats (values represent means ± SEM, *$p < .05$).

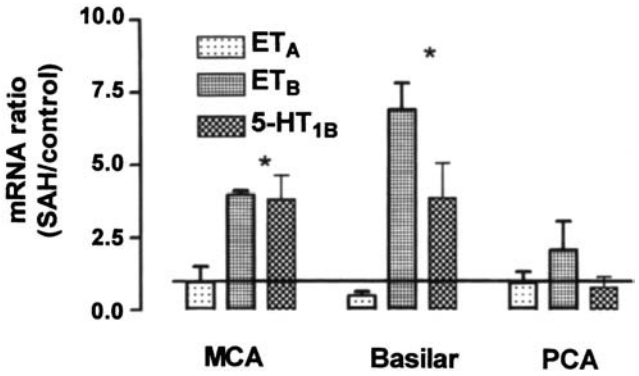


FIGURE 30–2 Bar graph showing the ratio of endothelin A (ET_A), endothelin B (ET_B), and 5-hydroxytryptamine $(5\text{-HT})_{1B}$ receptor messenger ribonucleic acid (mRNA) between rats with experimental subarachnoid hemorrhage and sham-operated rats. The number of copies of ET_B and 5-HT_{1B} receptor mRNA was increased in the arteries also exhibiting an increase in agonist sensitivity. Values represent means ± SEM (* $p < .05$). MCA, middle cerebral artery, PCA, posterior communicating artery.

properties of the cerebral arteries. More specifically, the sensitivity toward the endogenous agonists endothelin-1 and 5-HT, both ubiquitously present, is increased, making the arteries contract in response to much lower concentrations of these agonists. The data on mRNA suggest that this increase is brought about through the de novo expression of endothelin B (ET_B) and 5-HT_{1B} receptors, respectively. In the experimental series the posterior communicating artery did not undergo the same type of phenotypic changes as noted in the middle cerebral and basilar arteries. At this time no real explanation for this difference may be offered, except macroscopic observation that blood did not accumulate as much around the posterior communicating artery compared with the other arteries.

The series presented involves an experimental rat model of SAH, and care should therefore be exercised in extrapolating the data to the human setting. It has been shown that human cerebral arteries are able to change their endothelin receptor phenotype through organ culture[7] and also that the ET_B receptor is expressed in human cerebral arteries under pathological conditions.[8] These observations increase the likelihood that the changes observed in the rat model also occur after SAH in humans.

The development of cerebral vasospasm is the result of a series of events over time, starting with the hemorrhage itself and ending in the pathological contraction of the cerebral arteries. The receptor upregulation noted and the increased sensitivity may represent a functional end stage in this sequence of events. So far we have examined two receptor systems, but others, such as angiotensin receptors, may also be affected and contribute to the pathological condition. The involvement of several receptor systems could explain the lack of clinical effect of single receptor antagonists[3] because inhibiting one system would not have any influence on other receptors. Should the multiple receptor hypothesis hold true, treatment should then be directed at multiple receptors using several inhibitors. An alternative, and perhaps from a clinical point of view, a more attractive way would be to identify the signal transduction pathway mediating the upregulation and then attempt a pharmacological blockade. In a study designed to examine the role of protein kinases A and C in upregulation of receptors in organ culture, we found that inhibition of protein kinase A led to enhanced upregulation of the 5-HT_{1B} receptor, whereas inhibition of protein kinase C led to inhibition of ET_B receptor upregulation.[9] The results are not unanimous, but do provide impetus for further research into intracellular kinases pivotally involved in the pathological expression of vascular receptors.

REFERENCES

1. Hargreaves R, Beer M. 5-Hydroxytryptamine and its receptors. In: Edvinsson L, Krause D, eds. Cerebral Blood Flow and Metabolism. New York: Lippincott, Williams & Wilkins, 2002:283–294
2. Bradley SR, Pieribone VA, Wang W, Severson CA, Jacobs RA, Richerson GB. Chemosensitive serotonergic neurons are closely associated with large medullary arteries. Nat Neurosci 2002;5: 401–402
3. Wilkins RH. Attempts at prevention or treatment of intracranial arterial spasm: an update. Neurosurgery 1986;18:808–825
4. Hoel NL, Hansen-Schwartz J, Edvinsson L. Selective up-regulation of $5\text{-HT}_{1B/1D}$ receptors during organ culture of cerebral arteries. Neuroreport 2001;12:1605–1608
5. Hansen-Schwartz J, Hoel NL, Zhou M, Xu CB, Svengaard NA, Edvinsson L. Experimental SAH alters endothelin receptor phenotype in rat cerebral arteries. In: Macdonald RL, ed. Cerebral Vasospasm: Advances in Research and Treatment. New York: Thieme Medical Publishers; 2004:79–82.
6. Hansen-Schwartz J, Hoel NL, Xu CB, Svendgaard NA, Edvinsson L. Subarachnoid hemorrhage-induced upregulation of the 5-HT_{1B} receptor in cerebral arteries in rats. J Neurosurg 2003;99:115–120
7. Hansen-Schwartz J, Nordström CH, Edvinsson L. Human endothelin subtype A receptor enhancement during tissue culture via de novo transcription. Neurosurgery 2002;50:127–133
8. Hansen-Schwartz J, Szok D, Edvinsson L. Expression of ET_A and ET_B receptor mRNA in human cerebral arteries. Br J Neurosurg 2002;16:149–153
9. Hansen-Schwartz J, Svensson CL, Xu C-B, Edvinsson L. Protein kinase mediated upregulation of endothelin A, endothelin B and 5-hydroxytryptamine 1B/1D receptors during organ culture in rat basilar artery. Br J Pharmacol 2002;137:118–126

31

What Is the Key Factor Expressed in Human Spastic Arteries After SAH?

SHIGEKI ONO, M.D., TOMOHITO HISHIKAWA, M.D., MITSUHISA NISHIGUCHI, M.D., SHINSAKU NISHIO, M.D., KOJI TOKUNAGA, M.D., KENJI SUGIU, M.D., ISAO DATE, M.D.

Abstract

There may be significant differences between human and animal vasospastic arteries. The expression of many genes and proteins is altered in vasospastic arteries after subarachnoid hemorrhage (SAH) in animal models, including proliferative, inflammatory, heat shock, and apoptotic proteins. There are, however, few studies of messenger ribonucleic acid (mRNA) and protein expression in vasospastic human arteries. We investigated the expression of endothelial nitric oxide synthase (eNOS), inducible NOS (iNOS), nuclear factor-kappa B (NF-KB), IK B kinase (IKK), and caspase-3 in the cerebral arteries obtained from a patient who died of vasospasm. The anterior cerebral arteries that were confirmed by angiography to have vasospasm and the nonspastic basilar artery (used as a normal control artery) were studied. The arteries were harvested 2 days after confirmation of vasospasm. Tissues were dissected and examined for protein expression of eNOS, iNOS, IKK, and caspase-3 by immunohistochemical staining. Enzyme-linked immunosorbent assay (ELISA) and reverse transcriptase-polymerase chain reaction (RT-PCR) were performed for iNOS to detect iNOS expression in cerebrospinal fluid from patients with SAH and the autopsied arteries, respectively. Expression of eNOS was decreased in the spastic anterior cerebral arteries, whereas iNOS, IKK, and caspase-3 were upregulated in the outer layers of these same arterial walls. RT-PCR showed a marked increase in iNOS expression in the vasospastic arteries. In some SAH patients, iNOS was detected in cerebrospinal fluid. In summary, eNOS, which potentially mediates vasodilation, decreased in the human vasospastic arteries, and inflammatory and apoptotic proteins conversely increased in the outer layer of the same arteries. In addition, iNOS was detected even in the cerebrospinal fluid of some patients with SAH.

Although animal models of vasospasm have given important information about the pathogenesis of cerebral vasospasm, differences between human and animal vasospastic arteries may be significant. Many genes and proteins have been reported to have altered expression in vasospastic arteries after subarachnoid hemorrhage (SAH) in animal models, including proliferative, inflammatory, heat shock, and apoptotic proteins.[1,2]

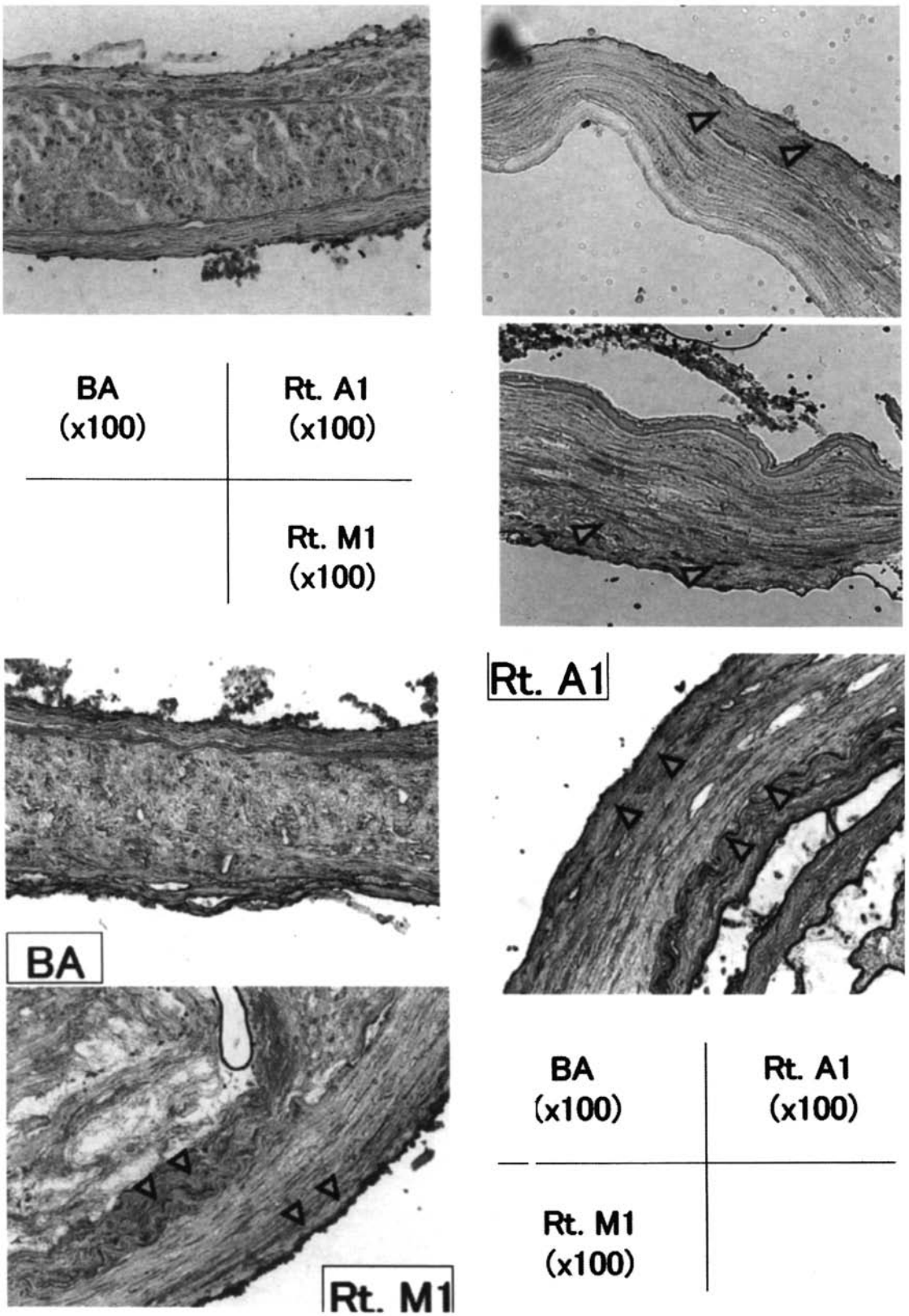
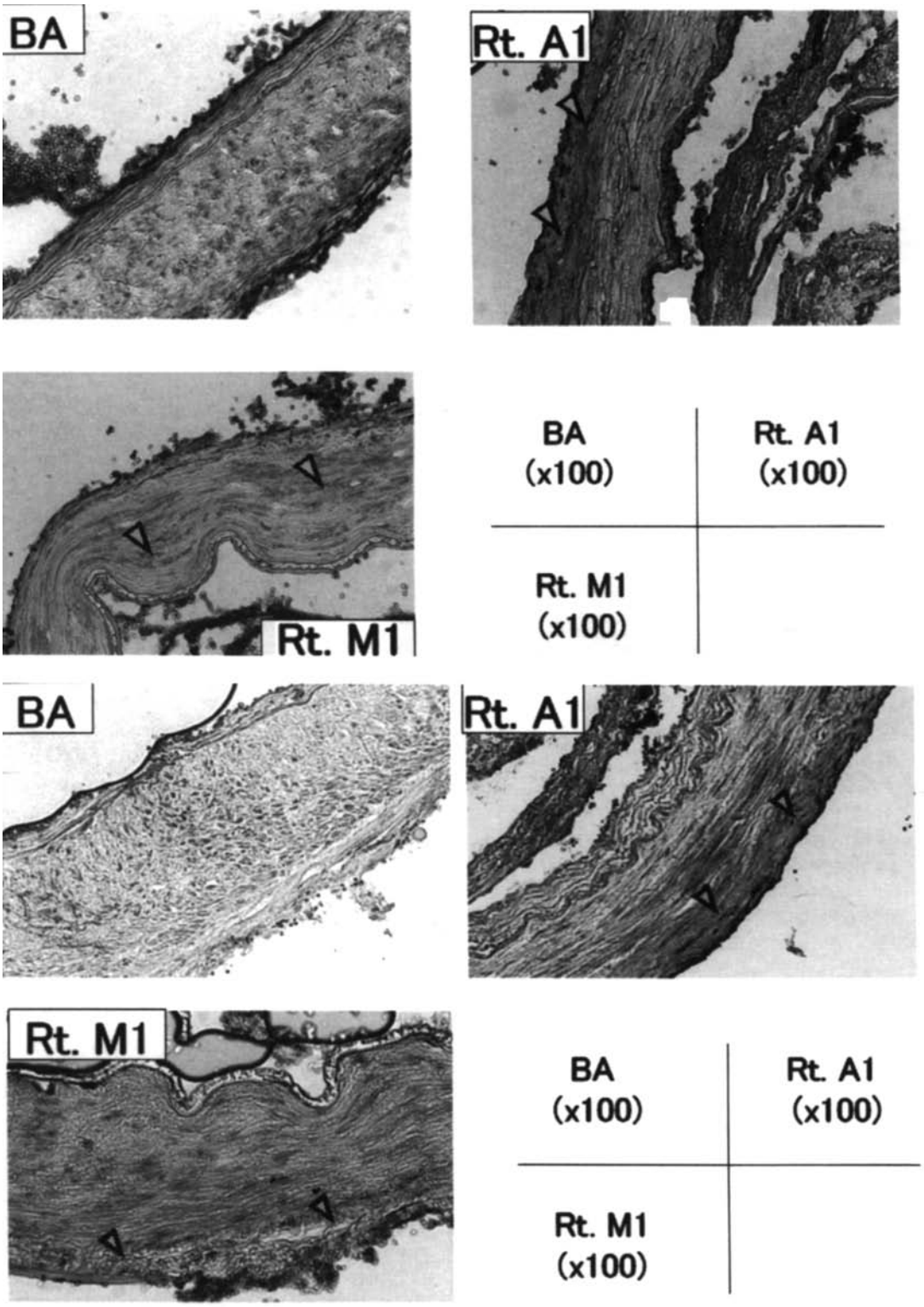


FIGURE 31–1 Photomicrographs of immiinohistochemistry for inducible nitric oxide synthase (iNOS) (upper left panels), nuclear factor-kappa B (NF-KB) (upper right panels), IK B kinase (IKK) (lower left panels), and caspase-3 (lower right panels). There was no expression of iNOS in the basilar artery (BA), whereas there was marked expression in both the right proximal anterior cerebral (A1) and middle cerebral (Ml) arteries, mainly in the outer layers of the vessels (arrowheads). Expression of NF-KB was detected in all layers of the basilar artery. In the right Al and Ml segments, positive cells were



detected in the outer layer of the vessels and also in the intima and subintima (arrowheads). IKK immunoreactivity was noted in the adventitial and intimal layers in the basilar artery. In the right Ml and A1, IKK was strongly expressed in the adventitial layer of the vascular wall with some expression in the tunica media (arrowheads). For caspase-3 immunoreactivity, there were only a few positive cells in the basilar artery, whereas in the vasospastic right Al and Ml there was immunoreactivity mainly in the adventitial areas of the vessels (all photomicrographs originally ×100 magnification).

However, there are few studies examining messenger ribonucleic acid (mRNA) and protein expression in vasospastic human arteries. This paucity of human data led us to investigate the expression patterns of endothelial nitric oxide synthase (eNOS), inducible NOS (iNOS), nuclear factor-kappa B (NF-KB), IK B kinase (IKK), and caspase-3 in vasospastic arteries obtained from a patient who died of SAH during vasospasm.

Clinical Materials and Methods

A 74-year-old man suffered from SAH and was admitted to Okayama University Hospital with a clinical Hunt and Kosnik grade of 3.[3] Emergent diagnostic angiography was performed, and an aneurysm located in the anterior communicating artery was identified. Embolization with Guglielmi detachable coils was performed at the time of angiography on the day of aneurysmal rupture. Eight days later, aneurysmal rehemorrhage occurred, and emergent angiography revealed vasospasm with more substantial vasospasm of the right-sided arteries. The patient died on day 11, and an autopsy was performed within 2 hours of death. Tissues were dissected quickly and examined for mRNA expression of iNOS by reverse transcriptase-polymerase chain reaction (RT-PCR) using TRIzol Reagent, Superscript (Invitrogen, Tokyo, Japan) and primers for human iNOS (sense: 5'-gaggaagtgggcaggagaatg-3', antisense: 5'-gtagtagaaaggggacaggac-3'). The protein expression of eNOS, iNOS, NF-KB, IKK, and caspase-3 were also examined by immunohistochemical staining (BD Biosciences, Palo Alto, CA, USA) using protocols suggested by the manufacturer. Enzyme-linked immunosorbent assay (ELISA) was performed for iNOS to confirm its expression in 15 samples of cerebrospinal fluid obtained from patients with SAH. This was done using a human iNOS ELISA kit (R&D systems, Minneapolis, MN, USA), and the protein absorbance was measured at 450 nm.

Results

Immunohistochemistry showed that eNOS expression was downregulated in the spastic anterior cerebral artery. On the other hand, iNOS, IKK, and caspase-3 were upregulated in the arterial outer wall layers of the anterior cerebral artery (Fig. 31–1). RT-PCR showed a marked increase in iNOS expression in the vasospastic artery (Fig. 31–2). In some SAH patients, iNOS was detected in the cerebrospinal fluid, whereas iNOS was not detected in normal cerebrospinal fluid, (Fig. 31–3). The highest amount of iNOS protein in cerebrospinal fluid was noted 5 days after SAH.

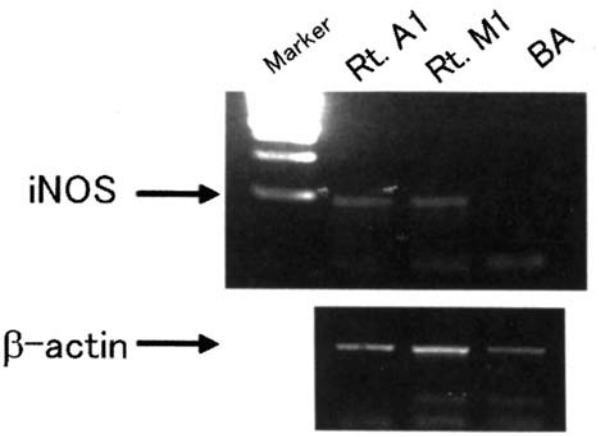


FIGURE 31–2 Reverse transcriptase polymerase chain reaction (RT-PCR) gels of inducible nitric oxide synthase (iNOS) messenger ribonucleic acid (mRNA) expression in the cerebral arteries after subarachnoid hemorrhage (SAH). RT-PCR showed marked iNOS mRNA expression in the spastic arteries (right A1 and Ml) whereas there was no expression of iNOS in the basilar artery (BA).

Discussion

The etiology of cerebral vasospasm is still unknown. One contributing factor may be the difficulty in obtaining and studying human arterial samples from the time when vasospasm occurs. Review of the literature of the histopathological analyses of vasospasm in humans shows there are few reports of what proteins change in cerebral arteries after SAH.[4,5] In addition, in the 16 reported autopsied cases in which the duration between angiographic vasospasm and death was specified, the arteries were obtained within 2 days of

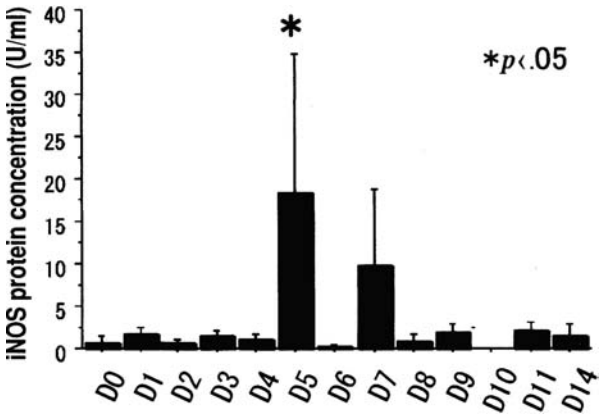


FIGURE 31–3 Bar graph showing results of enzyme-linked immunosorbent assay (ELISA) for inducible nitric oxide synthase (iNOS) protein in cerebrospinal fluid. The iNOS protein was detected in cerebrospinal fluid after SAH with a maximal at 5 days after subarachnoid hemorrhage (SAH) ($p < .05$ compared with normal cerebrospinal fluid).

diagnosis of angiographic vasospasm in only one case.[6] In this article, the expression of eNOS protein decreased in the endothelial cells of the spastic arteries compared with the control, nonspastic basilar artery This finding suggests that the endothelial cells in the major cerebral arteries may be damaged during vasospasm, as studies using experimental models of SAH have suggested. Regarding iNOS, our data showed expression of iNOS protein was mainly in the outer layers of the spastic arteries in humans. Inducible NOS is expressed in inflammatory cells that are present in the subarachnoid space as part of the inflammatory reaction that accompanies SAH. The role of iNOS in vasospasm, however, has been controversial.[7] From our results, we conclude that even if iNOS production is observed during vasospasm in the vascular wall, it may not cause enough cerebral vasodilation to overcome vasospasm after SAH. This may be due to the overexpression of cytokines or free radicals produced by iNOS such as peroxynitrite.

Regarding apoptosis, caspase-3 is a key enzyme involved in the execution of the intracellular apoptotic signaling pathway. In particular, the activation of caspase-3 cleaves several different proteins and is important for the initiation of apoptosis.[8] The expression of caspase-3 in the endothelial cells in the spastic arteries observed in the case presented here did not correlate with endothelial dysfunction as represented by the reduced expression of eNOS. Our results for caspase-3 expression imply that its expression pattern was quite similar with that of NF-KB, which is thought to be a key transcription factor in the inflammatory reaction. Knowledge about caspase-mediated apoptosis of endothelial cells after SAH is still rudimentary, however, and we consider that caspase-3 may be induced in the outer layer of the vasospastic vascular wall by several cytokines released from leukocytes or platelets after SAH. Immunocytochemical findings of NF-KB and IKK showed that NF-KB is expressed predominantly in the adventitial layer and subintimal tissue in which inflammatory cells are frequently observed. The location of IKK expression was quite similar to that of NF-KB. Therefore, NF-KB activity may be upregulated because it is regulated by IKK.

Limitations of this study should be acknowledged. Immunocytochemical examinations are not quantitative, and results may vary due to factors other than quantity of protein. The amount of arterial samples is small, and we therefore could not perform the quantitative analysis of those proteins using techniques such as Western blotting. Second, the results are based on the analysis of only one case. Statistical analysis could not be performed. Study of additional autopsy cases may reveal the importance of key factors controlling vascular tone after SAH.

Conclusion

We report a case that was autopsied soon after death due to SAH in which the vasospastic anterior cerebral arteries were available for examination. Immunocytochemical findings revealed decreasing of eNOS in the endothelial cells, which is also known to occur in experimental models of vasospasm. There was evidence for inflammatory and apoptotic reactions occurring in the subarachnoid space in and around the vasospastic arterial segments in this human case.

REFERENCES

1. Aihara Y, Kasuya H, Onda H, Hori T, Takeda J. Quantitative analysis of gene expressions related to inflammation in canine spastic artery after subarachnoid hemorrhage. Stroke 2001;32:212–217
2. Onda H, Kasuya H, Takakura K, et al. Identification of genes differentially expressed in canine vasospastic cerebral arteries after subarachnoid hemorrhage. J Cereb Blood Flow Metab 1999;19:1279–1288
3. Kosnik EJ, Hunt WE. Postoperative hypertension in the management of patients with intracranial arterial aneurysms. J Neurosurg 1976;45:148–154
4. Zubkov AY, Ogihara K, Bernanke DH, Parent AD, Zhang J. Apoptosis of endothelial cells in vessels affected by cerebral vasospasm. Surg Neurol 2000;53:260–266
5. Honma Y, Fujiwara T, Irie K, Ohkawa M, Nagao S. Morphological changes in human cerebral arteries after percutaneous transluminal angioplasty for vasospasm caused by subarachnoid hemorrhage. Neurosurgery 1995;36:1073–1080
6. Suzuki S, Kimura M, Souma M, Ohkima H, Shimizu T, Iwabuchi T. Cerebral microthrombosis in symptomatic cerebral vasospasm: a quantitative histological study in autopsy cases. Neurol Med Chir (Tokyo) 1990;30:309–316
7. Sayama T, Suzuki S, Fukui M. Role of inducible nitric oxide synthase in the cerebral vasospasm after subarachnoid hemorrhage in rats. Neurol Res 1999;21:293–298
8. DeGracia DJ, Kumar R, Owen CR, Krause GS, White BC. Molecular pathways of protein synthesis inhibition during brain reperfusion: implications for neuronal survival or death. J Cereb Blood Flow Metab 2002;22:127–141

32

Expression of Hypoxia Inducible Factor-1 in a Rat Subarachnoid Hemorrhage Model

TOMOHITO HISHIKAWA, M.D., SHIGEKI ONO, M.D., MITSUHISA NISHIGUCHI, M.D., SHINSAKU NISHIO, M.D., KOJI TOKUNAGA, M.D., KENJI SUGIU, M.D., ISAO DATE, M.D.

Abstract

Hypoxia inducible factor-1 (HIF-1) is a key transcription factor that regulates expression of many genes such as vascular endothelial growth factor, erythropoietin, and glucose transporters. This transcription factor is induced by hypoxia after cerebral ischemia and myocardial infarction. We examined HIF-1 expression at the level of messenger ribonucleic acid (mRNA) and protein after subarachnoid hemorrhage (SAH) in rats. We employed a single-injection SAH model in rats. Under general anesthesia, 0.4 mL of autologous arterial blood or saline was injected into the rat cisterna magna. Rats were sacrificed 6, 24, or 48 hours after SAH. mRNA was extracted immediately from the brain stem, and reverse transcriptase polymerase chain reaction was performed using HIF-1 specific primers. At the same time points, protein was obtained from the brain stem, and HIF-1 protein expression, examined by Western blot analysis using a polyclonal anti-rat HIF-1 a antibody. Immunohistochemistry also was done using the same antibody. The mRNA for HIF-1 was not changed significantly at any time. On the other hand, HIF-1 protein was increased 48 hours after SAH compared with the saline-injected group. HIF-1 protein expression increased gradually after SAH. HIF-1 positive cells were found in the ventrolateral side of the brain stem. In this model, HIF-1 expression peaked at the time of maximal vasospasm. These results suggest that exposure of the brain to subarachnoid blood can cause significant ischemic damage, and HIF-1 may have protective effects against vasospasm in this model.

Hypoxia inducible factor-1 (HIF-1) is a key transcription factor that has an important role in regulating oxygen homeostasis.[1] For example, HIF-1 regulates expression of many genes such as vascular endothelial growth factor (VEGF), erythropoietin, and glucose transporters.[2,3] HIF-1 is induced not only in ischemic brain[4,5] but also in the brain suffering from cerebral hemorrhage.[6] Moreover, there is evidence that HIF-1 protects neurons from injury. There are, however, no reports that mention the relationship between HIF-1 expression and cerebral vasospasm. This study examined HIF-1 expression at the level of messenger ribonucleic acid (mRNA) and protein after subarachnoid hemorrhage (SAH) in rats to determine effects of SAH on expression of this transcription factor. VEGF mRNA was also analyzed.

Materials and Methods

Animal Model

Male Sprague-Dawley rats weighing 250 to 300 g were divided into two groups to undergo a single intracisternal injection of blood or to be used as controls. Rats were placed under general anesthesia by intraperitoneal injection of sodium pentobarbital (1 mg/kg). Autologous arterial blood (0.4 mL, SAH group) or physiological saline (0.3 mL, control group) was injected into the cisterna magna. Rats were sacrificed 6, 24, or 48 hours after the injection and their brain stems were removed and stored in liquid nitrogen until tissue processing.

Reverse Transcription and Polymerase Chain Reaction

Total mRNA was extracted using TRIZOL Reagent (Invitrogen Life Technologies, Carlsbad, CA, USA) and complementary deoxyribonucleic acid was synthesized using a Superscript First-Strand Synthesis System (Invitrogen Life Technologies). Polymerase chain reaction (PCR) was performed with oligonucleotide primers for HIF-lα (sense: 5'-AAGTCTAGGGATGCAGCAC-3' and antisense: 5'-CAAGATCACCAGCATCTAG-3') VEGF (sense: 5'-TGCACCCACGACAGAAGGGGA-3' and antisense: 5'-TCACCGCCTTGGCTTGTCACAT-3') and glyceraldehyde-3-phosphate dehydrogenase (GAPDH, sense: 5'-TCCCTCAAGATTGTCAGCAA-3' and antisense: 5'-AGATCCACAACGGATACATT-3'). GAPDH was used as an internal control. The relative densities of bands were analyzed with National Institutes of Health Image (Version 1.61, Bethesda, MD, USA).

Western Blot Analysis

Protein (50 μg) from each brain stem was separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to a Hybond-P pure nitrocellulose membrane (Amersham Pharmacia Biotech, Little Chalfont, Buckinghamshire, England). Membranes were probed with 1:500 dilution of the primary antibody (mouse antihuman HIF-lα, Novus Biologicals, Littleton, CO, USA), β-actin was analyzed as an internal control. The relative densities of bands were analyzed with National Institutes of Health Image (Version 1.61).

Immunohistochemistry

Rats were anesthetized and perfused with phosphate-buffered saline and 4% paraformaldehyde. The brains were removed and stored in 4% paraformaldehyde overnight and then soaked in 30% sucrose for 3 days at 4°C. The brain stems were sectioned on a cryostat (10 μm sections). Polyclonal rabbit antihuman HIF-lα was used as the primary antibody (1:50 dilution, Santa Cruz Biotechnology, Santa Cruz, CA, USA). Hematoxylin staining was performed for counterstaining.

Statistical Analysis

Data are given as means ± standard errors of the means with significance at $p < .05$ for comparisons between groups by analysis of variance (ANOVA) followed by pairwise comparisons if significant variance occurred.

Results

Reverse transcription (RT)-PCR data showed that HIF-lα mRNA expression did not change significantly among SAH groups (Fig. 32–1). At 48 hours after SAH, HIF-lα mRNA was significantly upregulated in comparison with the control group ($p < .05$). The amount of VEGF mRNA was maximal 48 hours after SAH and was significantly higher at this time compared with the earlier times after SAH ($p < .05$).

Western blot analysis demonstrated that protein expression of HIF-lα was significantly upregulated 48 hours after SAH compared with the control group at the same time ($p < .05$). Moreover, among the SAH groups, HIF-lα protein expression gradually increased over time. The amount of HIF-lα protein 48 hours after SAH was significantly greater than the amount present 6 and 24 hours after SAH ($p < .05$, Fig. 32–2). Immunohistochemical study of brain stem cross sections demonstrated HIF-lα

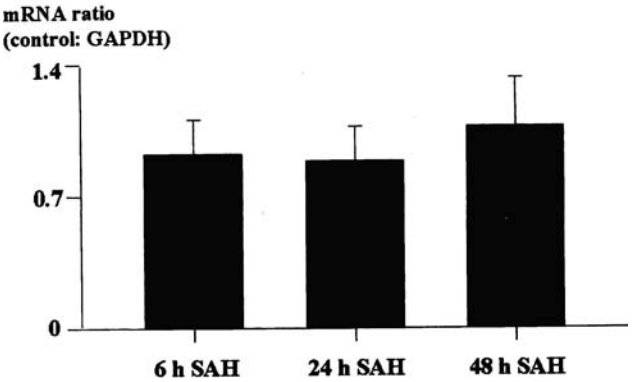


FIGURE 32–1 Bar graph showing semiquantitative analysis of hypoxia inducible factor-1 (HIF-l)α messenger ribonucleic acid (mRNA) in rats with subarachnoid hemorrhage (SAH). The amount of mRNA is expressed as a ratio of HIF-lα mRNA to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA at 6, 24, and 48 hours after SAH.

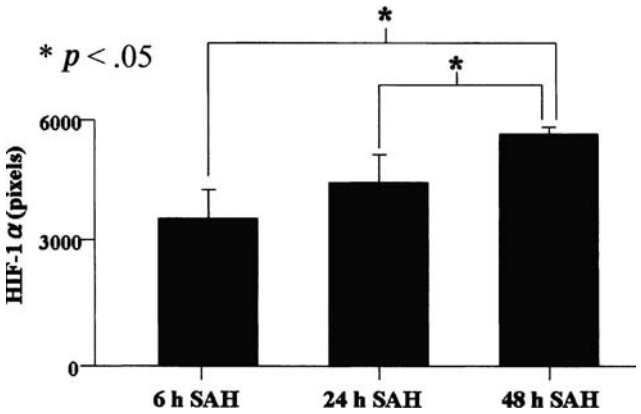


FIGURE 32–2 Bar graph of western blot analysis of hypoxia inducible factor-1 (HIF-l)α protein in rats with subarachnoid hemorrhage (SAH). The amount of HIF-lα protein was expressed as the relative density as analyzed with National Institutes of Health Image. There was a significant increase in HIF-lα protein with increased time after SAH ($p < .05$, ANOVA).

positive cells in the ventrolateral side of the brain stem 48 hours after SAH (Fig. 32–3). Control, saline-injected rats showed only sparse HIF-lα-positive cells in their brain stems.

Discussion

HIF-1 is a transcriptional complex that is emerging as a key mediator of oxygen homeostasis. HIF-1, composed of HIF-lα and HIF-1β subunits, is involved in development, pulmonary hypertension, ischemia, and tumorogenesis.[1] More than 40 genes that are regulated by HIF-1 have been identified, including erythropoietin, VEGF, and glucose transporter l.[7] Under normal oxygenation, HIF-lα is ubiquitinated by Hippel-Lindau tumor suppressor protein and, subsequently, degraded by the proteasome, leading to loss of its activity.[2,8] On the other hand, HIF-lα is stabilized under hypoxic conditions and is translocated into the nucleus, where it binds to its binding site on DNA and subsequently controls expression of many downstream genes. Regulation of the activation of HIF-1 is generally at the level of HIF-1 protein by mechanisms involving protein stabilization and destabilization as already mentioned.

This study examined the relationship between HIF-1 expression in the brain stem and SAH. SAH was associated with a slight but significant increase in the mRNA level 48 hours after SAH, a finding that is at odds with that reported by others.[6,8] Interestingly, changes in expression of HIF-lα protein expression were chronologically identical to those in expression of mRNA. In the rat SAH model, it is generally reported that there is an early phase of vasospasm 10 minutes after a single SAH and a more delayed phase occurring ~48 hours later. Therefore, these data suggest that brain stem ischemia due to vasospasm can somehow upregulate HIF-1 mRNA expression. An increase in mRNA and stabilization of HIF-1 protein under hypoxic conditions may induce the maximal expression of HIF-1 protein 48 hours after SAH. We also noted that mRNA expression of VEGF was maximal 48 hours after SAH.

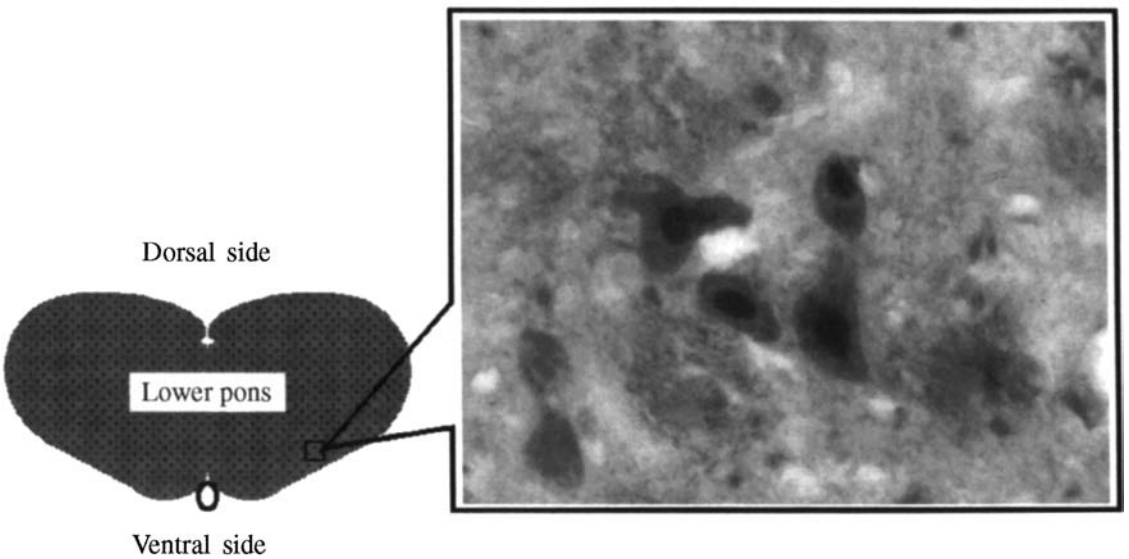


FIGURE 32–3 Drawing (left) of rat brain cross section showing the location from which the photomicrograph (right) was taken. Photomicrograph shows immunohistochemistry of HIF-lα protein in the rat brain stem 48 hours after subarachnoid hemorrhage.

We hypothesize that the HIF-1-VEGF pathway may be involved in cerebral ischemia due to vasospasm and that this pathway may be neuroprotective.

Immunohistochemical findings demonstrated that HIF-lα positive cells were found in the ventrolateral side of brain stem adjacent to where autologous blood was injected. This result supports the fact that the ischemic brain stem after SAH expresses HIF-1 even in the period of vasospasm. The main limitation of the preceding data must be acknowledged: no evidence is presented to document that vasospasm occurred or that there was any ischemia of the brain stem.

There are reports that the expression of HIF-1 protein has cytoprotective effects on brain tissue in intracerebral hemorrhage[6] and cerebral ischemia models.[4,5] Taking our data into consideration, it is reasonable at least to hypothesize that HIF-1 protein expression protects brain tissue from neuronal damage due to ischemia after SAH. We believe that HIF-1 could be a promising target for therapy of cerebral vasospasm after SAH.

REFERENCES

1. Maxwell PH, Pugh CW, Ratcliffe PJ. The pVHL-HIF-1 system: a key mediator of oxygen homeostasis. In: Roach RC, ed. Hypoxia: From Genes to the Bedside. New York: Kluwer Academic/Plenum; 2001:365–376
2. Ramirez-Bergeron DL, Simon MC. Hypoxia-inducible factor and the development of stem cells of the cardiovascular system. Stem Cells 2001;19:279–286
3. Semenza GL. Hypoxia-inducible factor 1: control of oxygen homeostasis in health and disease. Pediatr Res 2001;49:614–617
4. Jin XL, Mao XO, Nagayama T, Goldsmith PC, Greenberg DA. Induction of vascular endothelial growth factor and hypoxia inducible factor-la by global ischemia in rat brain. Neuroscience 2000;99:577–585
5. Chavez JC, LaManna JC. Activation of hypoxia-inducible factor-1 in the rat cerebral cortex after global ischemia: potential role of insulin-like growth factor-1. J Neurosci 2002;22:8922–8931
6. Yajun J, Jimin W, Richard FK, Ya H, Julian TH, Guohua X. Hypoxia-inducible factor-la accumulation in the brain after experimental intracerebral hemorrhage. J Cereb Blood Flow Metab 2002;22:689–696
7. Semenza GL. Signal transduction to hypoxia-inducible factor 1. Biochem Pharmacol 2002;64:993–998
8. Salceda S, Caro J. Hypoxia-inducible factor lα (HIF-lα) protein is rapidly degraded by the ubiquitin proteasome system under normoxic conditions. J Biol Chem 1997;272:22642–22647

33

Interactive Role of Protein Kinase C Isoforms and Rho Kinase in Vasospasm After Experimental Subarachnoid Hemorrhage

KAZUO OBARA, PH.D., SHIGERU NISHIZAWA, M.D., PH.D., MASAYO KOIDE, M.S., AYAKO MITATE, M.S., KOICHI NAKAYAMA, PH.D.

Abstract

We previously reported that the protein kinase C (PKC) isoforms, PKC δ and α, were involved in the pathogenesis of vasospasm after subarachnoid hemorrhage (SAH) in dogs. Other investigators, however, have suggested that Rho/Rho kinase and myosin light chain (MLC) phosphorylation also are important in the genesis of vasospasm in this model. The purpose of the present study was to clarify how PKC isoforms interact with Rho kinase in pathogenesis of cerebral vasospasm after SAH. A two-hemorrhage canine model was created, and the animals were treated with Y-27632, a Rho kinase inhibitor, and rottlerin, a specific inhibitor of PKC δ. The drugs were injected into the cisterna magna. Angiographic vasospasm, translocation of PKC δ and α and RhoA to the membrane fraction of arterial cells, and MLC phosphorylation levels in the basilar artery were examined. PKC δ translocated to the membrane fraction early after SAH, and this was followed by PKC α translocation. RhoA translocation and MLC phosphorylation increased on day 4 after the second blood injection and continued until day 7. Y-27632 inhibited vasospasm and PKC δ translocation on day 4 but did not affect PKC α translocation or the vasospasm on day 7. Y-27632 also inhibited MLC phosphorylation on days 4 and 7. Rottlerin attenuated vasospasm and PKC δ translocation on day 4 but did not affect vasospasm and PKC α translocation on day 7. Rottlerin had no effect on RhoA translocation and MLC phosphorylation on days 4 and 7. The results suggest that PKC δ activated by Rho kinase is responsible for the initiation of cerebral vasospasm, whereas PKC α may be involved in the maintenance of the vasospasm.

Cerebral vasospasm is one of the most serious complications of subarachnoid hemorrhage (SAH). It has profoundly deleterious effects on the cerebral circulation. In spite of extensive clinical and experimental studies, however, the pathophysiological and molecular mechanisms for cerebral vasospasm after SAH still remain to be clarified.[1] Regarding the pathophysiological mechanism of cerebral vasospasm, we demonstrated that the protein kinase C (PKC) isoforms PKC δ and α were involved in the dog model.[2] On the other hand,

Rho/Rho kinase and myosin light chain (MLC) phosphorylation have been suggested to play important roles in this pathological process.[3] The purpose of the present study was to clarify how PKC isoforms interact with Rho kinase in the pathogenesis of cerebral vasospasm after SAH in dogs.

Materials and Methods

The basilar arteries of beagle dogs of either sex weighing 7 to 19 kg were used. Vasospasm was created using the double-hemorrhage canine model as previously described.[4] We performed baseline angiography and a cisternal blood injection on day 1 and the second injection of blood on day 4. The animals were treated with rottlerin, an inhibitor of PKC δ or Y-27632, a Rho kinase inhibitor. Three mL sterile phosphate-buffered saline with or without rottlerin (10 μmol/L) or Y-27632 (10 μmol/L) was injected into the cisterna magna. Translocation of PKC isoforms and RhoA were measured by Western blot analysis performed by a modification of the procedure described by Obara et al.[5] Chronological changes in the level of MLC phosphorylation were quantified as described previously.[5]

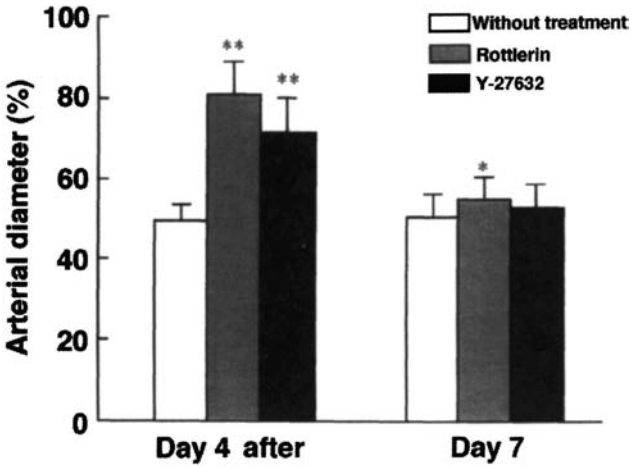


FIGURE 33–1 Bar graph showing effects of rottlerin and Y-27632 on angiographic arterial diameters after subarachnoid hemorrhage (SAH) in dogs. The diameter of the basilar artery on day 1 (control) was 1.21 ± 0.04 mm (n = 9). The diameter on each day was expressed as a percentage of the control (control = 100%). Data are expressed as the mean ± standard error of the mean of three experiments. There is significantly less vasospasm on day 4 after the second blood injection in dogs injected with rottlerin and Y-27632 compared with the control diameter on that day (* $p < .05$ and ** $p < .01$). On day 7, there is significantly less vasospasm after rottlerin injection compared with the control group without drug treatment.

Results and Discussion

Figure 33–1 shows chronological changes in angiographic diameter of the basilar artery in the two-hemorrhage canine model. In the control group, the diameter of basilar artery on day 1 (control) was 1.21 ± 0.04 mm (= 100%, n = 9). The vessel narrowed to 85% of its baseline diameter on day 4 before the second blood injection of autologous blood and narrowed further to almost half of the control diameter on day 4 after the second blood injection (initial phase). The vasospasm became significant after the second injection, and significant vasospasm remained on day 7 (late phase).

Rottlerin (5 μmol/L), an inhibitor of PKC δ and Y-27632 (5 μmol/L), a Rho kinase inhibitor, had no apparent effect on the basilar artery diameter on day 4 before the second blood injection (see Fig. 33–1). Rottlerin prevented the occurrence of vasospasm on day 4 after the second blood injection. Rottlerin also demonstrated a small but significant ability to reduce the degree of vasospasm on day 7. Y-27632 dilated the vasospastic artery on day 4 after the second injection but did not ameliorate vasospasm on day 7 (see Fig. 33–1). These results suggest that the development of vasospasm involves both PKC δ and Rho kinase pathways.

Figure 33–2 shows the chronological changes in translocation of PKC δ, PKC α, and RhoA assessed by Western blot analysis. In accordance with the progression of vasospasm, PKC δ was translocated initially on day 4 from the cytosol to the membrane fractions (see Fig. 33–2A), and PKC α was translocated on day 7 (see Fig. 33–2B). RhoA translocated from the cytosol to the membrane fraction on day 4 after the second blood injection, and this translocation persisted on day 7 (see Fig. 33–2C).

In the drug-treated groups, rottlerin (5 μmol/L) inhibited the translocation of PKC δ from the cytosol to the membrane fraction on day 4 after the second blood injection, and this inhibitory effect persisted on day 7. Y-27632 (5 μmol/L) attenuated the translocation of PKC δ from the cytosol to the membrane fraction on day 4 after the second blood injection but did not affect this translocation on day 7 (see Fig. 33–2A). Rottlerin and Y-27632 (each at 5 μmol/L), however, had no apparent effect on the translocation of PKC α (see Fig. 33–2B) and RhoA (see Fig. 33–2C) from the cytosol to the membrane fraction both on day 4 after the second blood injection and on day 7. These results suggest that the activation of PKC δ is mediated by Rho kinase in the initial phase of vasospasm.

Figure 33–3 shows the MLC phosphorylation pattern analyzed by Western blotting. On day 1 (control) and day 4 before the second blood injection, there were two bands showing immunoreactivity to the MLC

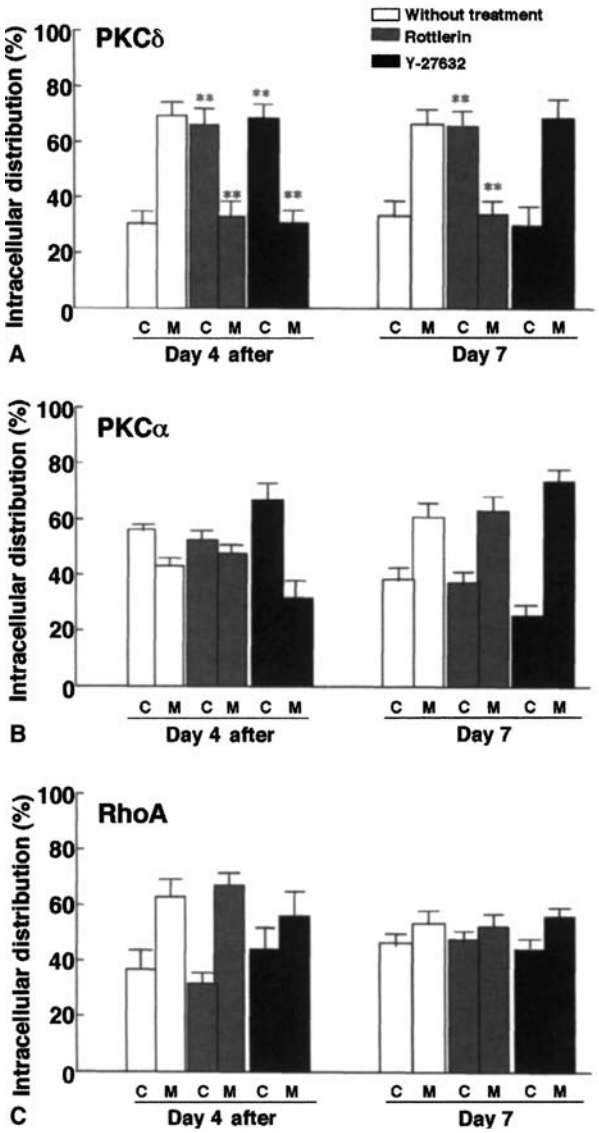


FIGURE 33–2 (A) Effects of rottlerin and Y-27632 on translocation of PKC δ, (B) PKC α, and (C) RhoA on day 4 after the second blood injection and on day 7. Three mL sterile phosphate-buffered saline with or without rottlerin (10 μmol/L) or Y-27632 (10 μmol/L) was injected into the cisterna magna. Bars represent means ± standard errors of the means of three experiments (** $p < .01$ compared with group without drug treatment). C, cytosol; M, membrane fraction.

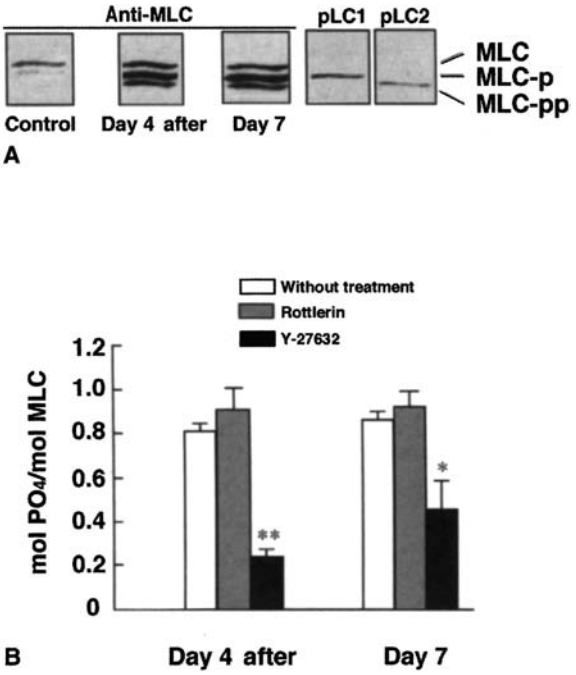


FIGURE 33–3 (A) Western blots showing 2 bands detected in control dog basilar artery, corresponding to myosin light chain (MLC) mono- and diphosphorylated forms as detected by the specific antibodies pLCl (detects phosphorylation at Ser-19) and pLC2 (detects phosphorylation at Ser-19/Thr-18). On day 4 after the second injection and day 7 a third band appears. (B) Bar graph showing effects of rottlerin and Y-27632 on MLC phosphorylation during vasospasm on day 4 after the second blood injection and on day 7. Bars represent means ± standard errors of the means of three experiments (* $p < .05$ and ** $p < .01$ compared with group without drug treatment).

antibody (anti-MLC, see Fig. 33–3A). On day 4 after the second blood injection and on day 7, an additional band was detected in addition to the previous two bands (see Fig. 33–3A). The top band was unrecognized by both the antibody specific for MLC monophosphorylated at Ser-19 (antibody pLCl) and for MLC diphosphorylated at Thr-18/Ser-19 (antibody pLC2). The second and third bands were recognized by antibodies pLC1 and pLC2, respectively (see Fig. 33–3A). These results indicate that monophosphorylated MLC20 (MLC-p) at Ser-19 and diphosphorylated MLC (MLC-pp) at Ser-19/Thr-18 increased on day 4 after the second blood injection and on day 7. Thus there was an overall increase in the total phosphorylation level of MLC. The total phosphorylation of MLC increased on day 4 after the second blood injection, and the high level of MLC phosphorylation was maintained throughout the course of vasospasm (see Fig. 33–3B). Rottlerin (5 μmol/L) had no effect on the high level of MLC phosphorylation on day 4 after the second blood injection or on day 7 (see Fig. 33–3B). On the other hand, Y-27632 (5 μmol/L) significantly decreased MLC phosphorylation on day 4 after the second blood injection and on day 7 (see Fig. 33–3B). These results suggest that MLC phosphorylation at Ser-19 and Ser-19/Thr-18 is mediated by Rho kinase and that this is important in the development of vasospasm after SAH in dogs.

In summary, the present results suggest that PKC δ is activated by Rho kinase and that this activation is

largely responsible for the initiation of cerebral vasospasm. There is a high level of MLC phosphorylation (at Ser-19 and Ser-19/Thr-18) during vasospasm and this is augmented by Rho kinase. PKC α may be involved in the maintenance of the vasospasm, which is functionally independent on MLC phosphorylation and Rho kinase activity.

REFERENCES

1. Janjua N, Mayer SA. Cerebral vasospasm after subarachnoid hemorrhage. Curr Opin Crit Care 2003;9:113–119

2. Nishizawa S, Obara K, Nakayama K, et al. Protein kinase C δ and α are involved in the development of vasospasm after subarachnoid hemorrhage. Eur J Pharmacol 2000;398:113–119

3. Wickman G, Lan C, Vollrath B. Functional roles of the Rho/Rho kinase pathway and protein kinase C in the regulation of cerebrovascular constriction mediated by hemoglobin. Relevance to subarachnoid hemorrhage and vasospasm. Circ Res 2003;92:809–816

4. Varsos VG, Liszczak TM, Han DH, et al. Delayed cerebral vasospasm is not reversible by aminophylline, nifedipine, or papaverine in a "two-hemorrhage" canine model. J Neurosurg 1983;58:11–17

5. Obara K, Hata S, Sato K, Koide M, Ishii K, Nakayama K. Contractile potentiation by endothelin-1 involves protein kinase C δ activity in the porcine coronary artery. Jpn J Physiol 1999;49:175–183

34

Possible Role of Heme Oxygenase-1 and Ferritin in Cerebral Vasospasm After Aneurysmal Subarachnoid Hemorrhage

HIDENORI SUZUKI, M.D., PH.D., KENJI KANAMARU, M.D., PH.D., MASATOSHI MURAMATSU, M.D., PH.D., KATSUHIRO TANAKA, M.D., HIROAKI FUJIWARA, M.D., PH.D., TADASHI KOJIMA, M.D., PH.D., WARO TAKI, M.D., PH.D.

Abstract

The goal of this prospective study was to clarify the potential role of an inducible heme-metabolizing enzyme, heme oxygenase (HO)-l, and an inducible iron-detoxifying protein, ferritin, in cerebral vasospasm after aneurysmal subarachnoid hemorrhage (SAH). The authors measured levels of bilirubin and iron, which are by-products of HO-1, and ferritin levels in the cerebrospinal fluid of 66 consecutive patients with aneurysmal SAH that was classified as Fisher grade 3 on computed tomography We determined the relation between these by-products of HO-1 and ferritin metabolism and vasospasm. Twenty-two of the 66 patients (33%) developed asymptomatic vasospasm and 16 patients (24%) developed symptomatic vasospasm. The levels of ferritin, bilirubin, and iron were all significantly elevated after SAH. The levels of ferritin and bilirubin were significantly higher in patients with no vasospasm than in patients with asymptomatic and symptomatic vasospasm on days 5 through 7 ($p < .01$ for ferritin; $p < 0.05$ for bilirubin) and on days 11 through 14 ($p < .025$ for bilirubin) after SAH. However, no significant difference was observed in the iron levels between these patient groups. This is the first study to show that higher levels of bilirubin and ferritin in the cerebrospinal fluid after SAH were associated with absence of vasospasm in the clinical setting. These findings support the concept that the induction of HO-1 and ferritin may be an intrinsic regulatory mechanism that acts to protect against cerebral vasospasm.

Subarachnoid hemorrhage (SAH) is removed from the subarachnoid space by intracranial metabolism and/or bulk drainage of cerebrospinal fluid (CSF). Routes of clearance include via the arachnoid villi although there probably are other routes.[1] Rapid evacuation of SAH may result in a decrease of spasmogens, leading to less severe vasospasm. Recent experimental studies showed that an inducible heme-metabolizing enzyme, heme oxygenase (HO)-l, was remarkably increased after SAH and mitigated delayed vasospasm by decreasing oxyhemoglobin and deoxyhemoglobin concentrations in CSF.[1] The ferrous iron component of oxyhemoglobin and deoxyhemoglobin may be responsible for cerebral vasospasm because free radical

formation and lipid peroxidation require the ferrous moiety, and ferrous iron is a potent trapping agent for the vasodilator nitric oxide. HO-1 releases free ferrous iron, which may contribute to vasospasm and stimulate the synthesis of an inducible iron-detoxifying protein, ferritin.[1,2] The increase in ferritin may be necessary to remove the potentially injurious ferrous iron. Ferritin-mediated iron detoxification following heme metabolism may also be important for the anti-vasospastic effect observed after the induction of HO-1, although other mechanisms are also possible. However, other recent experimental studies reported that another by-product of HO-1, bilirubin oxidation products, might contribute to vasospasm.[3] HO-1 and fer-ritin may also be a potential source of catalytic iron depending on conditions.[2] Thus, the induction of HO-1 and ferritin may either mitigate or aggravate cerebral vasospasm in the clinical setting. Because HO-1 acts intracellularly and is not known to be secreted extracellularly,[12] we measured iron, bilirubin, and ferritin levels in the CSF obtained from SAH patients to clarify the relation of these by-products of HO-1 or ferritin to vasospasm.

Clinical Materials and Methods

This prospective study included 66 consecutive patients (33 males and 33 females), 38 to 90 years of age (mean age of 61 years). Patients were included if they had aneurysmal SAH that was classified as Fisher grade 3 on admission cranial computed tomography (CT).[4] Patients were excluded if they developed an angiographic or surgical complication, if they did not undergo a stable xenon CT examination, or if there was motion artifact on the xenon CT scan. Clinical grading on admission was based on the World Federation of Neurosurgical Surgeons scale.[5] There were 18 patients evaluated as grade 1, 17 as grade 2, 9 as grade 3, 11 as grade 4, and 11 as grade 5. The ruptured aneurysm was located on the anterior communicating artery (n = 25), middle cerebral artery (n = 17), posterior communicating artery (n = 15), anterior choroidal artery (n = 2), basilar tip (n = 2), superior cerebellar artery (n = 2), posterior inferior cerebellar artery (n = 2), or anterior cerebral artery (n = 1). Surgical clipping (n = 60) or endovascular coiling (n = 6) of the ruptured aneurysm was performed within 48 hours of SAH. Cisternal (n = 17) or lumbar (n = 5) drainage was performed according to the preference of the attending neurosurgeon. All patients received intravenous fasudil hydrochloride beginning on the day after surgery and continuing until 14 days had elapsed from the time of SAH. A ventricular catheter was placed in all patients with symptomatic hydrocephalus (n = 4).

A xenon CT examination was performed at least once 7 to 9 days after SAH in all patients and was repeated when clinical examination or daily transcranial Doppler sonography suggested vasospasm. Two axial CT scan slices were examined for resting cerebral blood flow measurements. One slice included the basal ganglia, and the other included the bodies of the lateral ventricles. Vasospasm was defined as regional hypoperfusion (< 80% of cerebral blood flow) in a brain territory compared with an identical contralateral vascular territory. This was irrespective of clinical symptoms.

CSF samples were obtained from the cisternal (n = 17), lumbar (n = 5), or ventricular (n = 4) drain or via a lumbar puncture (n = 40) from 3 to 7 days after SAH. The sampling was performed one to three times in each patient, and the day for sampling was randomly selected. Control CSF samples were obtained from 10 patients with minimal cervical or lumbar spondylosis. The CSF concentrations of total protein and inflammatory cells were determined using an automatic chemistry analyzer. The CSF levels of iron, bilirubin, and ferritin were determined using quick-auto-neo-Fe (Shino-Test Corp., Tokyo, Japan) for iron, E-HR-Wako (Wako Pure Chemicals Industries, Ltd., Osaka, Japan) for bilirubin, and Immunoticles Auto-ferritin 2 (A & T Corp., Yokohama, Japan) for ferritin.

All values were expressed as means ± standard error of the mean. Comparisons between the two groups were made by unpaired t-tests. A probability value of < .05 was considered significant.

Results

Thirty-eight of 66 patients (58%) developed vasospasm that was asymptomatic in 22 cases (33%) and symptomatic in 16 (24%). The CSF levels of ferritin (1.47 ± 0.15 μg/mL), total bilirubin (0.40 ± 0.04 mg/dL), and iron (23 ± 2 μg/dL) were significantly elevated after SAH compared with the values for control patients (6.0 ± 0.9 ng/mL, $p < .005$, 0.060 ± 0.005 mg/dL, $p < .005$, and 2.3 ± 0.7, $p < .025$, respectively). The levels of ferritin and bilirubin were significantly higher in patients without vasospasm than in patients with vasospasm on post-SAH days 5 through 7 ($p < .01$ for ferritin and $p < .05$ for bilirubin) and on days 11 through 14 ($p < .025$ for bilirubin, Figs. 34–1 and 34–2). Ferritin increased in a delayed fashion in comparison with bilirubin. There was no significant difference observed in the levels of iron, total protein, and inflammatory cells between these patient groups (Figs. 34-3 to 34-5).

Discussion

This study showed that patients with no evidence of vasospasm after aneurysmal SAH had higher levels of

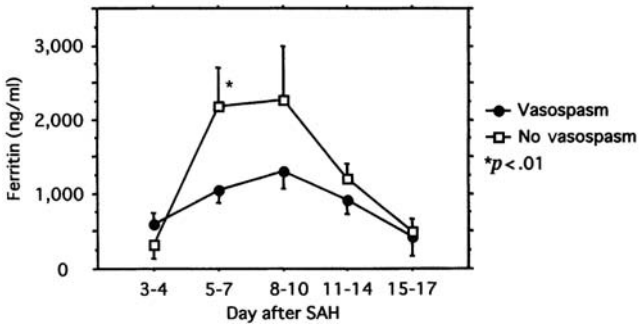


FIGURE 34–1 Ferritin concentrations in the cerebrospinal fluid of subarachnoid hemorrhage (SAH) patients by day after SAH. Values are means ± standard errors of the means. Patients are divided into those with vasospasm (n = 38) and those without vasospasm (n = 28). Patients with vasospasm had significantly lower levels of ferritin 5 to 7 days after SAH (p < .01).

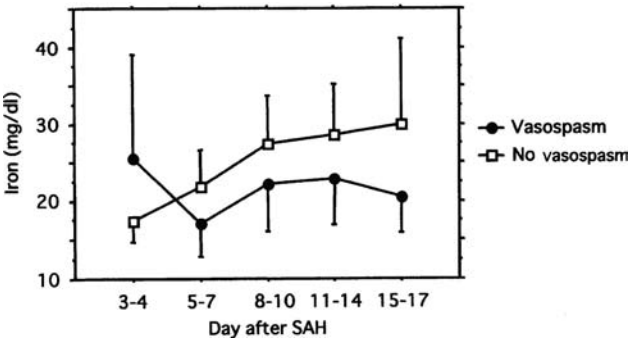


FIGURE 34–3 Iron concentrations in the cerebrospinal fluid of subarachnoid hemorrhage (SAH) patients by day after SAH. Values are means ± standard errors of the means. Patients are divided into those with vasospasm (n = 38) and those without vasospasm (n = 28). There were no significant differences between groups in iron levels at any time.

ferritin and bilirubin, a metabolite of HO-1, in the CSF. Both the volume of SAH, which was roughly estimated using CT scans, and the iron levels were not significantly different between patients with and without vasospasm. Although the method used to measure iron did not allow the differentiation of ferritin-bound iron from other non-heme-bound iron, ferritin-bound iron may resist cyclical reduction/oxidation reactions.[12] Therefore, the redox-active iron in patients who did not develop vasospasm might be significantly lower due to the greater ferritin-mediated iron detoxification. These findings support the concept that redox-active iron may be responsible for cerebral vasospasm and that the induction of HO-1 and ferritin may be an intrinsic regulatory mechanism that acts against vasospasm. This study also suggests that bilirubin itself may not cause vasospasm.

Inflammatory pial membrane, brain glial, and vascular cells may all play a role in metabolizing extracellular heme.[1] HO-1 plays a key role in recycling hemoglobin iron, and a link between iron release from the cells and HO-1 activity has been suggested.[2] Most iron may leave CSF by bulk drainage of CSF, being recycled to the blood plasma.[6] However, CSF in itself has a low iron-binding capacity that is close to saturation under normal conditions.[6,7] Although ferritin has a high iron-binding capacity, it is primarily localized intracellularly, and its concentration in CSF is very low.[7] Under pathological conditions such as SAH, the increase in ferritin that occurs not only detoxifies intracellular iron but is also secreted into the CSF, where it might be a primary detoxifying and delivery protein for iron.[7] In this study, the increase in ferritin was much greater than that of iron in the CSF after SAH, especially in patients without vasospasm. Vasospasm

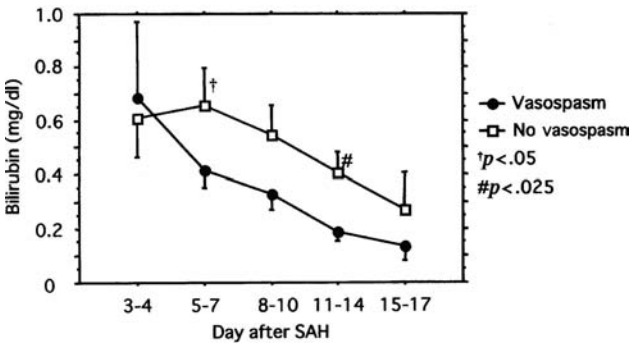


FIGURE 34–2 Bilirubin concentrations in the cerebrospinal fluid of subarachnoid hemorrhage (SAH) patients by day after SAH. Values are means ± standard errors of the means. Patients are divided into those with vasospasm (n = 38) and those without vasospasm (n = 28). Patients with vasospasm had significantly lower levels of bilirubin 5 to 7 and 11 to 14 days after SAH (p < .01).

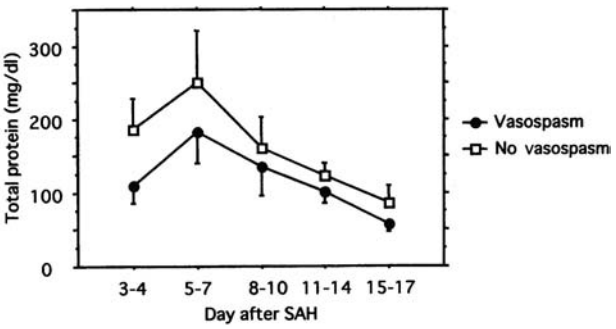


FIGURE 34–4 Total protein concentrations in the cerebrospinal fluid of subarachnoid hemorrhage (SAH) patients by day after SAH. Values are means ± standard errors of the means. Patients are divided into those with vasospasm (n = 38) and those without vasospasm (n = 28). There were no significant differences between groups in protein levels at any time.

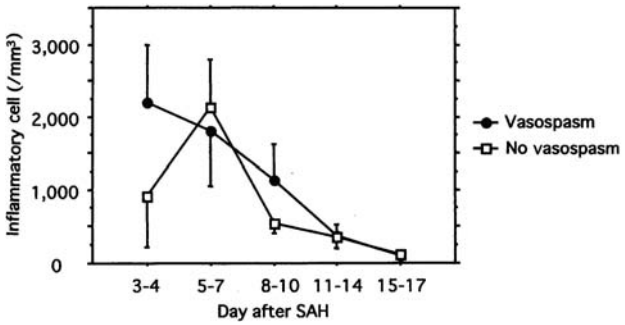


FIGURE 34–5 Number of inflammatory cells in the cerebrospinal fluid of subarachnoid hemorrhage (SAH) patients by day after SAH. Values are means ± standard errors of the means. Patients are divided into those with vasospasm (n = 38) and those without vasospasm (n = 28). There were no significant differences between groups at any time.

may not occur if HO-1 and ferritin are increased sufficiently such that they can preserve iron homeostasis after SAH.

REFERENCES

1. Suzuki H, Kanamaru K, Tsunoda H, et al. Heme oxygenase-1 gene induction as an intrinsic regulation against delayed cerebral vasospasm in rats. J Clin Invest 1999;104:59–66
2. Ryter SW, Tyrrell RM. The heme synthesis and degradation pathways: role in oxidant sensitivity: heme oxygenase has both pro- and antioxidant properties. Free Radic Biol Med 2000;28:289–309
3. Clark JF, Reilly M, Sharp FR. Oxidation of bilirubin produces compounds that cause prolonged vasospasm of rat cerebral vessels: a contributor to subarachnoid hemorrhage-induced vasospasm. J Cereb Blood Flow Metab 2002;22:472–478
4. Fisher CM, Kistler JP, Davis JM. Relation of cerebral vasospasm to subarachnoid hemorrhage visualized by computerized tomographic scanning. Neurosurgery 1980;6:1–9
5. Drake CG, Hunt WE, Sano K, et al. Report of World Federation of Neurological Surgeons Committee on a Universal Subarachnoid Hemorrhage Grading Scale. J Neurosurg 1988;68:985–986
6. Bradbury MWB. Transport of iron in the blood-brain-cerebrospinal fluid system. J Neurochem 1997;69:443–454
7. LeVine SM, Lynch SG, Ou CN, Wulser MJ, Tarn E, Boo N. Ferritin, transferrin and iron concentrations in the cerebrospinal fluid of multiple sclerosis patients. Brain Res 1999;821:511–515

35

Hypothermia Reduces Metabolic Alterations Caused by Acute Vasospasm After SAH in Rats: A Microdialysis and Magnetic Resonance Spectroscopy Study

CLAUDIUS F. C. THOME, M.D., GERRIT A. SCHUBERT, M.D., SVEN POLI, M.D., AMINIDAV MENDELOWITSCH, M.D., SABINE HEILAND, PH.D., LOTHAR SCHILLING, M.D., PETER SCHMIEDEK, M.D.

Abstract

Acute cerebral vasospasm has been shown to contribute significantly to acute brain injury after subarachnoid hemorrhage (SAH) and to be reduced by hypothermia. The purpose of this study was to investigate the effects of moderate hypothermia on the metabolic derangements following massive experimental SAH. SAH was induced in 46 anesthetized rats by injection of 0.5 mL arterial blood into the cisterna magna over 60 seconds. In 16 animals two microdialysis probes were implanted stereotactically in the frontoparietal cortex and samples were collected for measurement of glucose, lactate, and amino acids every 30 minutes for 3 hours following SAH in rats who were normothermic (37.0 ± 0.2°C) or hypothermic (32.0 ± 0.2°C). In 30 animals magnetic resonance spectroscopy was used for quantification of metabolite concentrations in normothermic and hypothermic rats and in rats where hypothermia was induced after SAH. SAH caused an initial reduction in glucose to 46 ± 13% in normothermic rats and to 76 ± 15% in hypothermic rats ($p < .01$). Hypothermia significantly reduced accumulation of lactate to 122 ± 18% compared with 206 ± 111% in the normothermic group ($p < .05$). The increase in glutamate (240 ± 162%) was abolished by hypothermia (133 ± 9%, $p < .05$), and the release of aspartate was significantly reduced (310 ± 88% in normothermic animals vs 207 ± 77% in the hypothermic group, $p < .05$). Comparable results were obtained for histidine ($p < .05$), γ-aminobutyric acid (not significant), and taurine (not significant). Glutamine was significantly increased in the hypothermia group. Magnetic resonance spectroscopy revealed that lactate accumulation was reduced from 244 ± 116% (normothermia) to 149 ± 34% (hypothermia, $p < .05$). Induction of hypothermia after SAH reduced peak lactate levels (186 ± 58%) and was associated with normalization of lactate levels within 3 hours. In conclusion, the acute phase of experimental SAH in rats was characterized by lactate accumulation and release of excitatory amino acids. Hypothermia reduced these changes significantly when induced before or after SAH.

The outcome after aneurysmal subarachnoid hemorrhage (SAH) is primarily dependent on the severity of the acute bleed, which is graded according to the acute neurological deficit of the patient. Aneurysm rupture causes a dramatic rise in intracranial pressure. This can reduce cerebral perfusion pressure and result in cerebral hypoperfusion. This phase of cerebral perfusion pressure-dependent acute global ischemia lasts only minutes.[1] Cerebral blood flow, however, remains depressed for hours, particularly in high-grade patients.[1,2] This cerebral perfusion pressure-independent hypoperfusion could be confirmed experimentally and has been shown to predict outcome.[3] The hypoperfusion has been suggested to be due to vasoconstriction or acute vasospasm and this phenomenon seems to contribute to brain injury in the acute phase of SAH.

These observations suggesting that there is ongoing injury for hours after SAH even when the cerebral perfusion pressure is normal raise the possibility that the outcome of SAH could be influenced favorably by neuroprotective measures in the acute stage. The authors have recently reproduced posthemorrhagic hypoperfusion due to acute vasospasm in a rat model of massive experimental SAH and tested the effect of moderate hypothermia.[4] Hypothermia reversed acute vasospasm even when induced after the insult. The effect of acute SAH, early vasosconstriction, and neuroprotective therapy on the brain parenchyma itself, however, remains unclear. The objective of this study, therefore, was to investigate the metabolic alterations that occur acutely following massive experimental SAH and to access the effect of hypothermia on these alterations.

Methods

Induction of SAH

All procedures were approved by the local animal care committee. Forty-six male Sprague-Dawley rats weighing ~300 g were divided into two groups. The groups were studied with microdialysis or magnetic resonance spectroscopy. The animals were artificially ventilated via a tracheostomy, and anesthesia was maintained with isoflurane. The right femoral artery was cannulated for monitoring of arterial blood pressure and blood gases. SAH was induced as previously described.[4] Briefly, blunt PE-10 tubing was advanced into the cisterna magna via an occipital burr hole, and 0.5 mL autologous, nonheparinized arterial blood was withdrawn from the femoral artery and injected into the subarachnoid space over 60 seconds.

Temperature Control

Temperature was monitored in the temporalis muscle and the esophagus to give an indication of pericranial and systemic body temperatures, respectively. Temperatures were monitored using a multichannel fiberoptic device (Luxtron, Santa Clara, CA, USA). Temperature was controlled by placing the rat in a hollow plastic spiral that was continuously perfused with water of the appropriate temperature. Experiments were performed at normothermia (37.0 ± 0.2°C), hypothermia (32.0 ± 0.2°C), or in rats that were cooled from 37 to 32°C just after SAH (secondary hypothermia). Blood gases were not corrected for actual body temperature (α-stat management).

Microdialysis

Microprobes (CMA 11, CMA Microdialysis AB, Solna, Sweden) were implanted stereotactically in the frontoparietal cortex and perfused with Perfusion Fluid CNS (CMA Microdialysis) at a rate of 1.5 µL/min. Samples were collected in normothermic and hypothermic animals every 30 minutes for 3 hours and analyzed for glucose, lactate, and amino acids using high-pressure liquid chromatography.

Magnetic Resonance Spectroscopy

The animals were placed in a 2.35 T Bruker Biospec 24/40 nuclear magnetic resonance scanner (Rheinstetten, GmbH). Volume selective spectroscopy (TR 2000 msec, TE 135 msec, 512 accumulations) was performed in a voxel with a size of ~0.4 cm^3. The voxel was positioned centrally in the rat brain. Metabolite spectra were phase corrected manually, and peak areas for choline and lactate were determined every 30 minutes for 3 hours. Lactate concentrations were semiquantitatively calculated using the lactate to choline ratio in normothermic, hypothermic, and secondary hypothermic animals.

Statistical Analysis

Data are presented as means ± standard deviations. Metabolite concentrations were converted and expressed as percent of baseline values. Differences between the experimental groups were evaluated by analysis of variance followed by paired and unpaired Student's *t*-tests and Bonferroni probabilities. Statistical significance was set at $p < .05$.

Results

SAH caused a prompt and highly significant fall in glucose concentrations in the cortical dialysate to a minimum of 46 ± 13% in normothermic rats. This decrease was markedly attenuated in hypothermic rats (76 ± 15%, $p < .01$). Hypothermia also significantly reduced accumulation of lactate to 122 ± 18% compared with 206 ± 111% in the normothermic

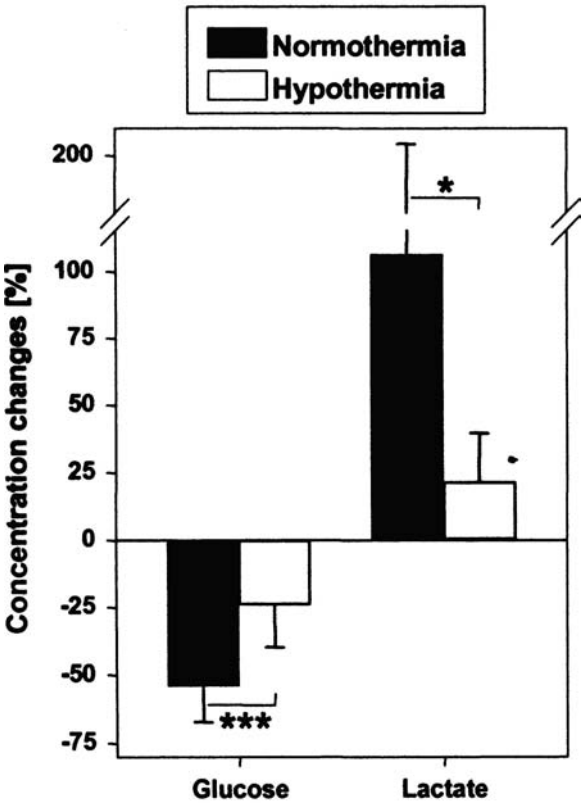


FIGURE 35–1 The maximal decrease of glucose and the maximal increase of lactate in the extracellular fluid within the first 3 hours after acute experimental subarachnoid hemorrhage in rats maintained normothermic or rendered hypothermic (values are means ± standard deviation, *: $p < .05$, ***: $p < .001$).

group ($p < .05$, Fig. 35–1). In normothermic animals, the excitatory amino acids glutamate and aspartate increased in the extracellular fluid early after SAH. Hypothermia abolished glutamate release (maximum of 133 ± 9% vs 240 ± 162% in normothermia group, $p < .05$) and markedly ameliorated aspartate release (maximum of 207 ± 77% vs 310 ± 88% in normothermia group, $p < .05$). Histidine, γ-aminobutyric acid, and taurine also significantly increased in normothermic rats whereas extracellular glutamine concentrations fell significantly after SAH ($p < .05$ compared with baseline values). Hypothermia minimized these changes, and mean concentrations did not differ from baseline. Peak values, however, were significantly different between normothermic and hypothermic rats for glutamine (Fig. 35–2).

Magnetic resonance spectroscopy in normothermic rats confirmed a global accumulation of lactate after SAH to a maximum of 244 ± 116%. This accumulation persisted throughout the study period and was significantly reduced by hypothermia (to a maximum of 149 ± 34%, $p < .05$). Cooling post-SAH (secondary hypothermia) reduced peak lactate levels (186 ± 58%, Fig. 35–3) and normalized them within 3 hours.

Discussion

Massive experimental SAH causes cerebral perfusion pressure-dependent global ischemia due to intracranial hypertension. This lasts for only a few minutes.[4,5] Following this is a second stage that lasts several hours and is characterized by vasoconstriction-induced hypoperfusion.[1,3–5] The clinical importance of hypoperfusion early after SAH is unknown but has been shown to correlate with clinical grade and outcome.[2] The present work shows that in our rat model the hypoperfusion leads to reduced delivery of glucose and is accompanied by lactate accumulation. This pattern also has been described in patients with SAH,[6] and lactate has been identified as the most sensitive marker of cellular energy imbalance after SAH.[7] Release of excitatory amino acids has been implicated in the deleterious

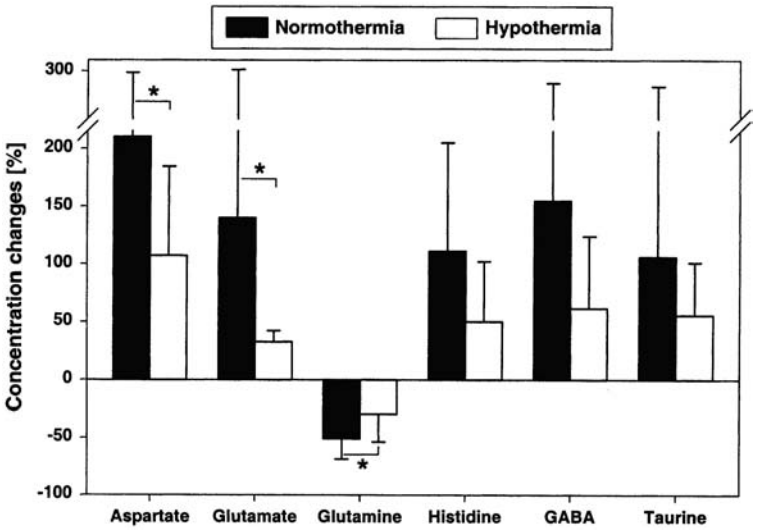


FIGURE 35–2 The maximal changes in extracellular amino acid concentrations within the first 3 hours after acute experimental subarachnoid hemorrhage in rats maintained normothermic or rendered hypothermic (values are means ± standard deviation, *$p < .05$).

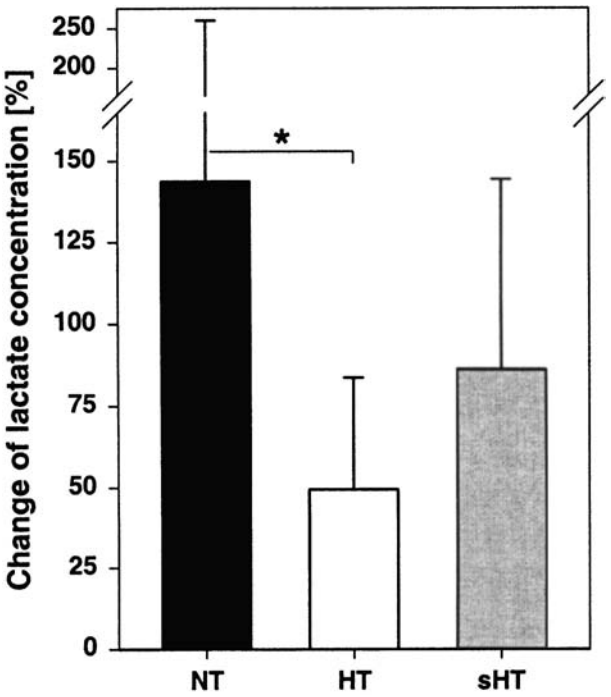


FIGURE 35–3 Bar graph demonstrating the maximal increase of lactate in the extracellular fluid within the first 3 hours after acute experimental subarachnoid hemorrhage (SAH). Note the decreased accumulation in the group subjected to hypothermia before SAH (HT) compared with normothermic rats (NT) and to rats made hypothermic after SAH (sHT, values are means ± standard deviation, $*p < .05$).

pathophysiological cascade after ischemic stroke and was found to correlate with unfavorable outcome[8] and ischemic metabolism[9] after clinical SAH. According to our microdialysis data, the release of glutamate, aspartate, histidine, and taurine is not only relevant for delayed posthemorrhagic ischemia but also takes place early after SAH. These changes may provoke initial brain injury and prevent patient recovery.

Our group has demonstrated previously that moderate hypothermia diminishes acute hypoperfusion and cerebral edema after experimental SAH.[4,10] Interestingly, short-lasting intraoperative hypothermia was able to reduce cerebral blood flow impairment for several days in SAH patients.[11] The present data show that hypothermia normalizes disturbed energy metabolism and increased neurotransmitter release in the acute stage of the disease, even if induced after the insult. These findings support the hypothesis that early neuroprotective treatment of SAH patients may have a marked impact on outcome.

Conclusion

The acute phase of massive experimental SAH in the rat is characterized by reduction of glucose, accumulation of lactate, and release of excitatory amino acids. These changes coincide with previously documented hypoperfusion due to acute vasospasm. Hypothermia normalizes the disturbed energy metabolism and reduces neurotransmitter release. The neuroprotective effect of hypothermia is also present if induced after the insult. These findings suggest a potential use of neuroprotective measures early after aneurysmal SAH.

REFERENCES

1. Jakobsen M. Role of initial brain ischemia in subarachnoid hemorrhage following aneurysm rupture: a pathophysiological survey Acta Neurol Scand Suppl 1992;141:1–33
2. Rosenstein J, Suzuki M, Symon L, Redmond S. Clinical use of a portable bedside cerebral blood flow machine in the management of aneurysmal subarachnoid hemorrhage. Neurosurgery 1984;15:519–525
3. Bederson JB, Levy AL, Ding WH, et al. Acute vasoconstriction after subarachnoid hemorrhage. Neurosurgery 1998;42:352–360
4. Thome C, Schubert G, Piepgras A, Elste V, Schilling L, Schmiedek P. Hypothermia reduces acute vasospasm following SAH in rats. Acta Neurochir Suppl 2001;77:255–258
5. Bederson JB, Germano IM, Guarino L. Cortical blood flow and cerebral perfusion pressure in a new noncraniotomy model of subarachnoid hemorrhage in the rat. Stroke 1995;26:1086–1091
6. Cesarini KG, Enblad P, Ronne-Engstrom E, et al. Early cerebral hyperglycolysis after subarachnoid haemorrhage correlates with favourable outcome. Acta Neurochir (Wien) 2002;144:1121–1131
7. De Micheli E, Pinna G, Alfieri A, et al. Postoperative monitoring of cortical taurine in patients with subarachnoid hemorrhage: a microdialysis study. Adv Exp Med Biol 2000;483:595–603
8. Staub F, Graf R, Gabel P, Kochling M, Klug N, Heiss WD. Multiple interstitial substances measured by microdialysis in patients with subarachnoid hemorrhage. Neurosurgery 2000;47:1106–1115
9. Schulz MK, Wang LP, Tange M, Bjerre P. Cerebral microdialysis monitoring: determination of normal and ischemic cerebral metabolism in patients with aneurysmal subarachnoid hemorrhage. J Neurosurg 2000;93:808–814
10. Piepgras A, Elste V, Frietsch T, Schmiedek P, Reith W, Schilling L. Effect of moderate hypothermia on experimental severe subarachnoid hemorrhage, as evaluated by apparent diffusion coefficient changes. Neurosurgery 2001;48:1128–1134
11. Karibe H, Sato K, Shimizu H, Tominaga T, Koshu K, Yoshimoto T. Intraoperative mild hypothermia ameliorates postoperative cerebral blood flow impairment in patients with aneurysmal subarachnoid hemorrhage. Neurosurgery 2000;47:594–599

SECTION V

Experimental Treatments

36

Prevention of Experimental Cerebral Vasospasm by Intrathecal Delivery of Liposomal Fasudil

YOSHIHIRO TAKANASHI, M.D., PH.D., TATSUHIRO ISHIDA, PH.D., JOHN H. ZHANG, M.D., PH.D., ISAO YAMAMOTO, M.D.

Abstract

We investigated the safety and efficacy of a sustained release form of liposomal fasudil for the prevention of cerebral vasospasm after experimental subarachnoid hemorrhage (SAH) in rats and dogs. The safety of a large intrathecal dose of liposomal fasudil was tested in 18 rats. Rats were divided into one of three groups. Each group received either 2.5 mg/kg or 5 mg/kg of liposomal fasudil or drug-free liposomes after SAH. Next, experimental SAH was induced in 15 dogs by injection of autologous arterial blood into the cisterna magna twice following baseline vertebral angiography. In six animals, 0.94 mg/kg of liposomal fasudil was injected into the cisterna magna (treatment group). In four animals, drug-free liposomes were similarly injected (placebo group), and the remaining five animals were treated with no liposomal injection after SAH (control group). On day 7 after SAH, angiography was repeated and cerebrospinal fluid was collected before sacrifice. In the safety study in rats, histological examination of the brains revealed no abnormalities. In the placebo and control groups, significant vasospasm occurred in the canine basilar artery on day 7. In the treatment group, vasospasm on basilar artery was significantly ameliorated ($p < .01$). More than 90% of fasudil was released from the liposomes into the cerebrospinal fluid. In conclusion, local delivery of liposomal fasudil is a safe and effective strategy for preventing vasospasm on experimental SAH.

Intrathecal drug therapy for cerebral vasospasm following subarachnoid hemorrhage (SAH) has some advantages over systemic delivery and may be more efficacious than systemic application.[1–3] In the current study, we have devised a sustained-release form of fasudil (liposomal fasudil) that can be used intrathecally and can continuously release the drug for several days. We investigated the safety and efficacy of liposomal fasudil in a sustained-release form for the prevention of cerebral vasospasm after experimental SAH.

Materials and Methods

Safety of Liposomal Fasudil

Preparation of liposomes was done as described in detail elsewhere.[1,2,4] Eighteen Sprague-Dawley rats were divided into one of three experimental groups. Experimental SAH was produced in all rats by two injections of autologous blood into the cisterna magna.[5] Two hours after the second blood injection the animals received either 2.5 mg/kg of liposomal

fasudil (n = 6), 5 mg/kg of liposomal fasudil (n = 6), or drug-free liposomes (n = 6) injected into the cisterna magna. Seven days after the initial blood injection, the brains were removed for histological examination.

Canine SAH Model

Experimental SAH was induced in 15 dogs by injection of autologous arterial blood into the cisterna magna twice. Vasospasm was assessed by comparision of vertebral angiograms taken at baseline and 7 days after the first SAH. In six animals, 0.94 mg/kg of liposomal fasudil was injected into the cisterna magna (treatment group). In four animals, drug-free liposomes were similarly injected (placebo group), and the remaining five animals were treated with no liposomal injection after SAH (control group). On day 7 angiography was repeated, and cerebrospinal fluid was collected before sacrifice. The percent change in basilar artery diameter was calculated by dividing the diameter of the basilar artery observed on the angiogram 7 days after SAH by that of the control diameter obtained from the baseline angiogram.

Results

Release of Liposomal Fasudil

Ninety percent of the fasudil was released into the cerebrospinal fluid of dogs by 5 days. Studies in vitro showed that, in contrast, 69% of the fasudil was released from liposomes incubated in control cerebrospinal fluid. As expected, no fasudil was detectable in blood samples.

Safety Study

On gross examination the brain, vessels, and meninges of all rats appeared normal. Light microscopy of the brain parenchyma, ependyma, vessels, and basal meninges appeared histologically normal.

Changes in Basilar Artery Diameters

Liposomal fasudil, at the nontoxic dose of 0.94 mg/kg, significantly prevented vasoconstriction in the canine basilar artery when compared with that of the control group and the placebo group (Fig. 36–1).

Discussion

Although a therapeutic concentration of drug can be administered directly into the cerebrospinal fluid, with less total drug required than with systemic administration in many cases, the intrathecal route has drawbacks to adaptation for clinical use. The time during which drug concentrations remain in the therapeutic window may be short even with intrathecal delivery. Additional difficulties are the technical problems associated with intrathecal administration (complications associated with the prolonged presence of external catheters), risk of infection and bleeding, potential adverse effects on intracranial pressure, and the theoretical concern that drug distribution may be adversely affected in the patient with SAH. Therefore, with regard to drug concentration, frequent or continuous drug infusion may be needed to maintain a therapeutic drug concentration in the cerebrospinal fluid, the use of which is hampered clinically by the factors already cited.

To overcome some of these disadvantages, various methods of sustained local drug delivery have been introduced for the treatment of experimental vasospasm. Inoue et al showed that intrathecal implantation of a slow-release tablet containing calcitonin gene-related peptide prevents vasospasm following SAH in monkeys.[6] Shiokawa and colleagues used a prolonged-release pellet of papaverine that could be implanted intracranially at the time of surgery and

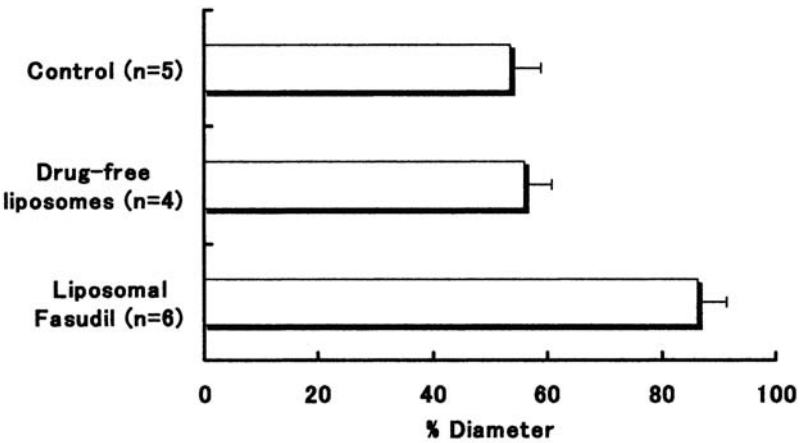


FIGURE 36–1 The diameter of the dog basilar artery 7 days after subarachnoid hemorrhage (SAH), expressed as a percent of the diameter observed on angiography before SAH on day 0. Bars represent means ± standard deviations. Treatment with liposomal fasudil significantly reduced the narrowing of the basilar artery on day 7 (* $p < .01$ vs drug-free group and control group).

reported that this prevented vasospasm in dogs.[7] Both methods require craniotomy to implant intracranially because the drug was contained in a solid form. On the contrary, liposomal fasudil that was used in this study is a liquid that can be delivered intracranially at the time of surgery or at other times by lumbar puncture. This might have the advantage of diffuse distribution of the drug throughout the entire neuraxis.

An additional advantage of liposomal fasudil might be the prolonged half-life of the drug that may result from slow release from the liposomes. This would have the advantage that a single intrathecal injection of liposomal fasudil might achieve a therapeutic drug concentration in the cerebrospinal fluid and prevent cerebral vasospasm, thus avoiding the need for frequent or continuous drug infusion. The results obtained from the current study may be an intriguing first step in applying the concept of sustained local drug delivery to the treatment of cerebral vasospasm in the clinical setting.

REFERENCES

1. Takanashi Y, Ishida T, Kirchmeier MJ, Shuaib A, Allen TM. Neuroprotection by intrathecal application of liposome-entrapped fasudil in a rat model of ischemia. Neurol Med Chir (Tokyo) 2001;41:109–115
2. Takanashi Y, Ishida T, Meguro T, Kirchmeier MJ, Allen TM, Zhang JH. Intrathecal application with liposome-entrapped fasudil for cerebral vasospasm following subarachnoid hemorrhage in rats. J Clin Neurosci 2001;8:557–561
3. Thomas JE, Rosenwasser RH, Armonda RA, Harrop J, Mitchell W, Galaria I. Safety of intrathecal sodium nitroprusside for the treatment and prevention of refractory cerebral vasospasm and ischemia in humans. Stroke 1999;30:1409–1416
4. Ishida T, Takanashi Y, Doi H, Yamamoto I, Kiwada H. Encapsulation of an antivasospastic drug, fasudil, into liposomes, and in vitro stability of the fasudil-loaded liposomes. Int J Pharm 2002;232:59–67
5. Suzuki H, Kanamaru K, Tsunoda H, et al. Heme oxygenase-1 induction as an intrinsic regulation against delayed cerebral vasospasm in rats. J Clin Invest 1999;104:59–66
6. Inoue T, Shimizu H, Kaminuma T, Tajima M, Watabe K, Yoshimoto T. Prevention of cerebral vasospasm by calcitonin gene-related peptide slow-release tablet after subarachnoid hemorrhage in monkeys. Neurosurgery 1996;39:984–990
7. Shiokawa K, Kasuya H, Miyajima M, Izawa M, Takakura K. Prophylactic effect of papaverine prolonged-release pellets on cerebral vasospasm in dogs. Neurosurgery 1998;42:109–116

37

Magnesium and Cerebral Vasospasm

GAIL J. PYNE-GEITHMAN, D.PHIL.,
SHINSUKE NAKAYAMA, M.D., D.PHIL.,
THOMAS A. D. CADOUX-HUDSON, M.D., PH.D.,
JOSEPH F. CLARK, PH.D.

Abstract

Magnesium (Mg^{2+}) is known to dilate vascular smooth muscle that has been contracted by various contractile agonists. This study set out to determine whether Mg^{2+} could be used to prevent or reverse vasospasm caused in vitro by the cerebrospinal fluid (CSF) removed from patients with vasospasm after subarachnoid hemorrhage (SAH). Oxygen consumption and isometric force measurements of the porcine carotid artery were used to assess the contractile and metabolic status of the vessels following stimulation by vasospastic CSF and the effect of manipulating Mg^{2+} (as $MgCl_2$) on these responses. Mg^{2+} caused a dose-dependent decrease in tension following contraction generated by CSF from patients with vasospasm. The rate of relaxation after a stretch (control; 16.1 ± 4.9 Nm/2 sec) was significantly decreased in the presence of CSF from patients with vasospasm. Relaxation was normalized after loading tissue with Mg^{2+}, 12 mmol/L (2.7 ± 0.7 vs 15.8 ± 4.2 Nm/2 sec). Tissue loaded with 12 mmol/L Mg^{2+} had a significantly decreased rate of oxygen consumption in the presence of CSF from patients with vasospasm (0.71 ± 0.03 vs 0.46 ± 0.08 mmol 0_2/min/g). These results suggest that Mg^{2}+ is a potent vasodilator that helps to normalize contractile behavior and metabolism of the porcine carotid artery exposed to CSF from patients with vasospasm.

Cerebral vasospasm after subarachnoid hemorrhage (SAH) from a ruptured aneurysm is a well-studied form of vasospasm although the mechanism of the vasoconstriction has yet to be elucidated.[1–3] Treatments targeted to the cerebral blood vessels have not, so far, proven to be effective in producing cerebral vasodilation or a reversal of vasospasm.[4] Cerebral vasospasm occurs 4 to 7 days after the initial hemorrhage in around 40% of the patients who survive the hemorrhage.[5] Despite the putative treatment window between the hemorrhage and the onset of vasospasm (3 days), there are, as yet, no effective therapies available to dilate the cerebral vessels of these patients.[4] Ram et al reported that topical application of Mg^{2+} to the basilar artery or intravenous delivery of Mg^{2+} significantly reversed vasospasm in a rat model of SAH.[6] The effect, however, was transient and vasospasm recurred as soon as the Mg^{2+} was removed. Boet and Mee reported promising results in patients with SAH to whom a 20 mmol bolus followed by continuous infusion of 84.7 mmol per day of Mg^{2+} was administered.[7] This dose resulted in a doubling of serum Mg^{2+} levels. Therapy was not started until 2 to 3 days posthemorrhage. Based in part on these data, we hypothesize that acute (and/or chronic) administration of Mg^{2+} will normalize the contractile and metabolic changes in the porcine carotid artery induced by vasospastic cerebrospinal fluid using our in vitro model of cerebral vasospasm after SAH.[8]

Methods

These have been previously published.[9] Briefly, to model SAH-induced cerebral vasospasm in vitro, cerebrospinal fluid (CSF) from vasospastic patients was obtained and treated as described in our previously published work.[4,8,9] CSF was characterized as vasospastic (CSF_v) or nonvasospastic (CSF_n) as described previously based on the ability of a 1 in 30 dilution of the CSF to stimulate O_2 consumption to ≥ 0.4 μmol/min/g dry weight.[4] Oxygen consumption rates above 0.4 μmol/min/g dry weight were classified as CSF_v and the CSF that did not stimulate O_2 consumption as CSF_n.

Results

Chronic Magnesium Effects

Loading the tissue with Mg^{2+} (estimated to contain 1.2 mmol/L intracellular free Mg^{2+})[10] had a significant ($p < .05$) effect on the maximum rate of O_2 consumption in response to CSF_v (stimulated from 0.27 ± 0.06 to 0.46 ± 0.10 |xmol/min/g dry weight) compared with the condition in the absence of CSF_v (Fig. 37–1). The percent increase in the rate of oxygen consumption, however, was not significantly different.

Acute Mg^{2+} Effects

Figure 37–2A shows the relationship between the concentration of Mg^{2+} in the organ bath and relaxation of tissue precontracted with KCl, 70 mmol/L or CSF_v. Each curve is the average of six separate dose-response curves performed on tissue from six different pigs. In both cases, a dose-response curve with an overall relaxation of 56% was observed. Figure 37–2B shows the effect of acutely adding Mg^{2+}, 12 mmol/L, to tissue that had been contracted with CSF_v as well as the lack of effect of rinsing off the CSF from the tissue treated with CSF_v. The reduction in tension upon the addition of Mg^{2+}, 12 mmol/L in the presence of CSF_v-induced tension is 16.6 ± 1.5 mN/mm ($n = 3$). Figure 37–3 shows the effect of Mg^{2+} on tissue contracted with CSF_v. The maximal contraction to CSF_v was reduced by 26% when a bolus of Mg^2+, 12 mmol/L, was added. This reduction is greater, reaching 35%, when the tissue is preloaded with Mg^2+, 12 mmol/L before the addition of CSF_v and even greater still (50%) when the Mg^2+ preloaded, CSF_v contracted tissue is then exposed to an acute bolus of Mg^{2+}, 12 mmol/L.

Discussion

Although there have been several studies describing partial reversal of vessel spasm with topical or intravenous application of Mg^{2+}, these have been phenomenological, and there have, until here, been no attempts to elucidate the mechanism of protection/reversal.[6,7,11] Pretreating the vascular smooth muscle with Mg^{2+} lowers the baseline respiration of the porcine carotid artery, In addition, the rate of O_2 consumption of the CSF_v-stimulated, Mg^{2+}-loaded tissue was lowered as well. Nitric oxide-mediated pathways are not likely to be involved because neither adenylate cyclase nor guanylate cyclase is associated with Mg^{2+}-induced relaxation.[12] The observation that the dose-response curves for relaxation by Mg^{2+} of tissue contracted with KCl, 70 mmol/L and CSF_v overlay each other (Figure 37–2A) may indicate Ca^{2+}-antagonistic activities of Mg^{2+}.

The stimulation of a slow onset of pathological constriction/failure to relax by CSF_v has so far proven to be resistant, at least in the vasospasm patient, to conventional treatments.[13] Mg^{2+} caused a relaxation (see Fig. 37–2B), which may be indicative of vasodilation, as well as protecting the metabolism, by decreasing

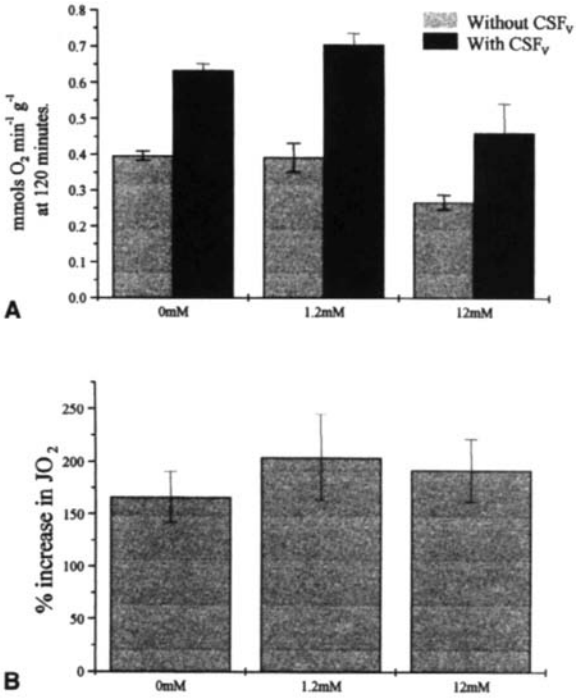


FIGURE 37–1 (A) Rates of O_2 consumption of the porcine carotid artery at 120 minutes without or with exposure to vasospastic cerebrospinal fluid (CSF_v), under exposure to different concentrations of Mg^{2+} (0 mM, $n = 6$; 1.2 mM, $n = 8$; 12 mM, $n = 6$). Values are means ± standard deviation (*$p < .05$ compared with condition without CSF_v). (B) Percent increase in O_2 consumption between baseline and CSF_v-stimulated porcine carotid arteries. Values are means ± standard deviation (0 mM, $n = 6$; 1.2 mM, $n = 8$; 12 mM, $n = 6$). There were no significant differences between groups.

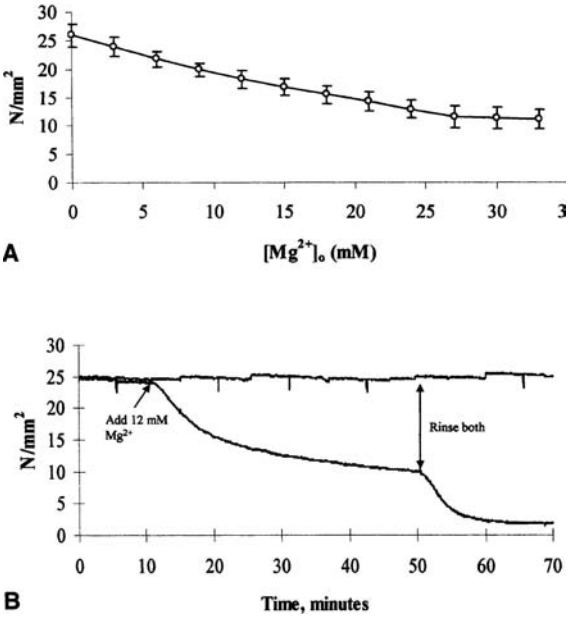


FIGURE 37–2 (A) Dose response curve of Mg^{2+} relaxation of the porcine carotid artery contracted with KCl,70 mmol/L (n = 6, values represent means ± standard deviations). (B) Representative trace of porcine carotid contracted with vasospastic cerebrospinal fluid (CSF_v). The precontracted tissue exposed to Mg^{2+} relaxed initially and then completely upon rinsing. Rinsing had no effect on the tissue not exposed to Mg^{2+}. This has been repeated at least five times.

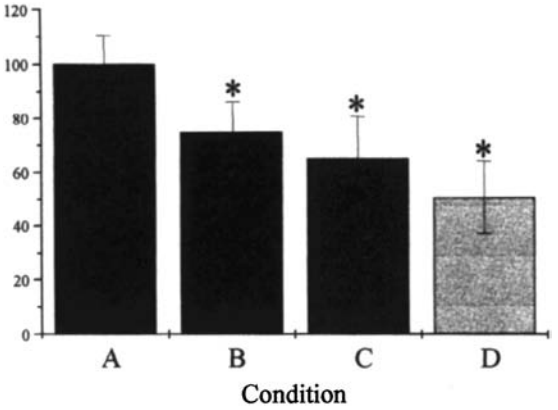


FIGURE 37–3 (A) Chronic and acute effects of Mg^{2+} (12 mmol/L) on pig carotid artery contracted with vasospastic cerebrospinal fluid (CSF_v). The loading of the tissue was performed in the organ bath at 37°C. Bars are contraction elicited by CSF_v under control Mg^{2+} (1.2 mmol/L) conditions and set as 100%, and (B) percent of that contraction achieved when Mg^{2+}, 12 mmol/L was added to the tissue contracted with CSF_v. (C) The percent of the CSF_v-induced contraction achieved when the tissue is preloaded with Mg^{2+}, 12 mmol/L and then rinsed before addition of CSF_v. (D) The percent of the CSF_v-induced contraction achieved when the tissue is preloaded with Mg^{2+}, 12 mmol/L and then exposed to CSF_v in the presence of the Mg^{2+}, 12 mmol/L. Bars are means ± standard deviations and n = 5 (different patient CSF samples) for each group (* indicates significant [$p < .05$] difference from control).

the rate of O_2 consumption of the porcine carotid artery. This may also protect the mitochondria from demands placed on it by the contractile apparatus. Ca^{2+} antagonism by Mg^{2+} could result in a lowered rate of respiration due to decreased Ca^{2+} stimulation of the mitochondria.[14] Another way in which Mg^{2+} may affect the mitochondria is that the mitochondria need an optimum Mg^{2+} concentration to function normally.[15] Mg^{2+} antagonism of Ca^{2+} is a likely candidate in this case because the tissue change in O_2 consumption in response to CSF_v remained constant despite the loading or depletion of intracellular Mg^{2+} (see Fig. 37–1B). We believe this also suggests that the stimulation of respiration by CSF_v is not exclusively Ca^{2+} dependent because the absolute rate of respiration, but not the relative increase, was decreased in the presence of Mg^{2+}.

Figure 37–3 suggests that both intracellular (bar C) and extracellular (bar B) Mg^{2+} may protect the vessels from stimulation by CSF_v. Smooth muscle contraction is initiated by the binding of Ca^{2+} to calmodulin and the subsequent binding of this Ca^{2+}–calmodulin complex to myosin light chain kinase. This would result in a lower tension and therefore lower O_2 consumption. The results in Figure 37–3 also corroborate the findings of Boet and Mee in that an acute bolus of Mg^{2+} in conjunction with tissue loading provides the most effective protection.[7] The Ca^{2+} antagonist activity of Mg^{2+} may not be the sole mechanism of protection because clinical trials involving Ca^{2+} channel blockers have not shown prevention or reversal of vasospasm.[16] Ca^{2+} channel blockers and N-methyl-D-aspartate receptor antagonists, including Mg^{2+}, may, however, have neuroprotective actions.[16,17]

Conclusion

These data suggest that Mg^{2+} therapy in vitro can relax vascular smooth muscle that has been contracted in response to CSF from patients with vasospasm, as well as protect the metabolism of the arteries. This may lead to investigation of the possible benefits of Mg^{2+} therapy in the patient with SAH. There is some evidence presented for the first time here that, although acute application of either topical or intravenous Mg^{2+} can elicit vasodilation in arteries contracted as described here, normal smooth muscle function is more effectively restored in vitro by first loading the tissue with Mg^{2+}. This study suggests that Mg^{2+} therapy may be more

effective if the patients with SAH had intravenous Mg^{2+} administered as a preventative measure to protect against vasospasm, rather than after the onset of vasospasm.

Acknowledgments

This research was funded by the Medical Research Council (MRC) of Great Britain, the MRC Collaborative Centre, and a Sasakawa Foundation Travelling Fellowship. The author wishes to thank Dr. Shinsuke Nakayama of the University of Nagoya Medical School for his useful discussion of the data included in this chapter.

REFERENCES

1. Endo S, Suzuki J. Experimental cerebral vasospasm after subarachnoid hemorrhage: development and degree of vasospasm. Stroke 1977;8:702–707
2. Macdonald RL, Weir BK. Cerebral vasospasm and free radicals. Free Radic Biol Med 1994;16:633–643
3. Weir B. The pathophysiology of cerebral vasospasm. Br J Neurosurg 1995;9:375–390
4. Cadoux-Hudson T, Pyne GJ, Clark JF. Subarachnoid hemorrhage induced cerebral vasospasm: a subcellular perspective on the control of tension. Emerg Ther Targets 1999;3:439–452
5. Weir B, Grace M, Hansen J, Rothberg C. Time course of vasospasm in man. J Neurosurg 1978;48:173–178
6. Ram Z, Sadeh M, Shacked I, Sahar A, Hadani M. Magnesium sulfate reverses experimental delayed cerebral vasospasm after subarachnoid hemorrhage in rats. Stroke 1991;22:922–927
7. Boet R, Mee E. Magnesium sulfate in the management of patients with Fisher grade 3 subarachnoid hemorrhage: a pilot study. Neurosurgery 2000;47:602–607
8. Pyne GJ, Cadoux-Hudson TA, Clark JF. The presence of an extractable substance in the CSF of humans with cerebral vasospasm after subarachnoid haemorrhage that correlates with phosphatase inhibition. Biochim Biophys Acta 2000;1474:283–290
9. Pyne GJ, Cadoux-Hudson TA, Clark JF. Cerebrospinal fluid from subarachnoid haemorrhage patients causes excessive oxidative metabolism compared to vascular smooth muscle force generation. Acta Neurochir (Wien) 2001;143:59–62
10. Nakayama S, Tomita T. Regulation of intracellular free magnesium concentration in the taenia of guinea-pig caecum. J Physiol 1991;435:559–572
11. Muir KW, Lees KR. A randomized, double-blind, placebo-controlled pilot trial of intravenous magnesium sulfate in acute stroke. Stroke 1995;26:1183–1188
12. White RE, Hartzell HC. Magnesium ions in cardiac function: regulator of ion channels and second messengers. Biochem Pharmacol 1989;38:859–867
13. Varsos VG, Liszczak TM, Han DH, et al. Delayed cerebral vasospasm is not reversible by aminophylline, nifedipine, or papaverine in a "two-hemorrhage" canine model. J Neurosurg 1983;58:11–17
14. Poe M. Kinetic studies of temperature changes and oxygen uptake in a differential calorimeter: energy balance during calcium accumulation by mitochondria. Arch Biochem Biophys 1969;132:377–387
15. Sloane BF, Scarpa A, Somlyo AP. Vascular smooth muscle mitochondria: magnesium content and transport. Arch Biochem Biophys 1978;189:409–416
16. Pickard JD, Murray GD, Illingworth R, et al. Effect of oral nimodipine on cerebral infarction and outcome after subarachnoid haemorrhage: British aneurysm nimodipine trial. BMJ 1989;298:636–642
17. Heath DL, Vink R. Magnesium sulphate improves neurologic outcome following severe closed head injury in rats. Neurosci Lett 1997;228:175–178

38

Phosphodiesterase III Inhibitor for the Treatment of Chronic Cerebral Vasospasm in Dogs

MITSUHISA NISHIGUCHI, M.D., SHIGEKI ONO, M.D., TOMOHITO HISHIKAWA, M.D., SHINSAKU NISHIO, M.D., KOJI TOKUNAGA, M.D., KENJI SUGIU, M.D., ISAO DATE, M.D.

Abstract

The smooth muscle cells of cerebral arteries contain a large amount of phosphodiesterase (PDE). Milrinone inhibits cyclic adenosine monophosphate–specific PDE III in both cardiac and vascular muscle. Vasodilation occurs because of the increase in cyclic adenosine monophosphate in vascular smooth muscle, facilitating Ca^{2+} uptake into the sarcoplasmic reticulum and reducing the amount of Ca^{2+} available for contraction and thus reducing vascular tone. Although there are some reports that intra-arterial or intravenous injection of milrinone may reduce vasospasm, the time course of the effect of and most effective route for administration of milrinone against vasospasm has not been reported. The present study investigated the effect of intra-arterial or intracisternal injection of milrinone on chronic experimental cerebral vasospasm in dogs. A double-hemorrhage canine model of vasospasm was used. After cerebral angiography was performed on days 0 and 7 and angiographic vasospasm was documented, milrinone was administrated intracisternally (0.1 mg) or intra-arterially (0.3 mg/kg/10 min). Angiography was performed 30, 60, 120, 180, 240, 300, and 360 minutes later, and the diameter of the basilar artery was measured. The degree of angiographic vasospasm was reduced with intracisternal injection of milrinone compared with baseline diameter on day 0 (66% at just before administration, 101 at 30, 105 at 60, 98 at 120, 91 at 180, 83 at 240, 74 at 300, and 74% at 360 minutes later). On the other hand, the degree of vasospasm was not reduced as effectively with intraarterial injection (57% at just before administration, 72 at 30, 77 at 60, 74 at 120, 78 at 180, 69 at 240, 64 at 300, and 63% at 360 minutes later). These results show that intracisternal injection of milrinone was more effective than intra-arterial injection at reversing established vasospasm in a canine model, at least in the doses tested. The effect, however, was transient and vasospasm recurred more than 180 minutes after injection.

Cerebrovascular smooth muscle contains a large amount of phosphodiesterase (PDE) with particularly abundant expression of PDE III. PDE inhibitors are vasodilators that mediate their effects by increasing the intracellular concentration of cyclic adenosine monophosphate (cAMP). This facilitates Ca^{2+} uptake into the sarcoplasmic reticulum, reduces free intracellular Ca^{2+}, and reduces vascular tone.[1] Although there have been reports that intra-arterial or intravenous injection of the PDE III inhibitor, milrinone, can reduce cerebral vasospasm, questions remain as to the most effective route of administration and the duration of action of the drug following single-dose treatments.[2–4] The present study investigated the effect of intra-arterial or intracisternal injection of milrinone on chronic cerebral vasospasm using a subarachnoid hemorrhage (SAH) model in dogs.

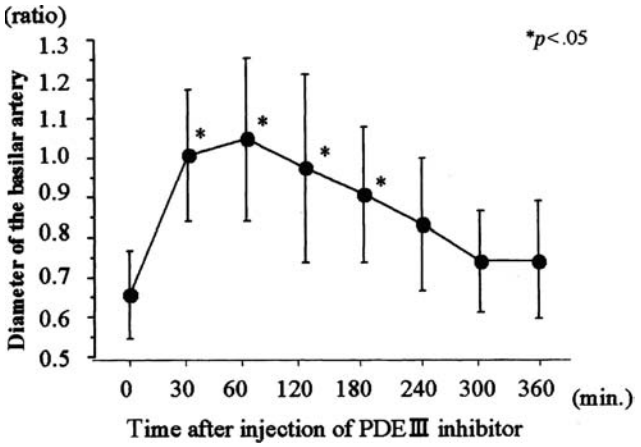


FIGURE 38–1 The effect of intracisternal injection of milrinone, a phosphodiesterase (PDE) III inhibitor, into the cisterna magna of dogs 7 days after subarachnoid hemorrhage (SAH). Diameter of the basilar artery is expressed as a ratio of the diameter on day 7 to that on day 0. Injection of milrinone resulted in significant dilation of the basilar artery 30, 60,120, and 180 minutes later ($p < .05$).

Materials and Methods

A double-hemorrhage canine model was used. Mongrel dogs weighing from 7 to 14 kg underwent injection of autologous, nonheparinized arterial blood (0.7 mL/kg) into the cisterna magna on day 0 and day 2. The effects of milrinone were examined by intracisternal or intra-arterial injection and using isolated basilar artery rings in an isometric tension study. For assessment of the effect of intracisternal injection, dogs were anesthetized with ketamine and sodium pentobarbital. An angiographic catheter was inserted through the right femoral artery. Angiography was performed on day 0 before SAH and repeated on day 7. After repeat angiography, milrinone (0.1 mg) was injected intracisternally, and angiography was repeated after 30, 60, 120, 180, 240, 300, and 360 minutes. The diameter of the basilar artery was measured to assess the degree of vasodilation achieved. For assessment of intra-arterial injection, angiography was performed as already described. Milrinone, 0.3 mg/kg/10 minutes was injected intra-arterially. Angiography was repeated after injection at the times previously noted and the basilar diameter measured.

Effects of milrinone were tested in vitro by obtaining ring segments from the basilar arteries of the dogs after sacrifice on day 7. Rings were precontracted with KCl, 60 mmol/L, and then milrinone was added in cumulative concentrations and the degree of relaxation was measured.

In an additional experiment using the double-hemorrhage canine model, dogs were euthanized on day 7, 90 minutes after injection of milrinone intracisternally or intraarterially. Angiography was performed prior to sacrifice and after milrinone injection to confirm the effect of the milrinone. Control animals included dogs subjected to the double-hemorrhage model but without milrinone treatment and normal dogs without SAH. All dogs were perfused transcardially with phosphate-buffered saline, 0.01 mol/L, followed by 4% paraformaldehyde. The brains were removed and the basilar arteries studied by histology (hematoxylin and eosin) and immunohistochemistry for cAMP.

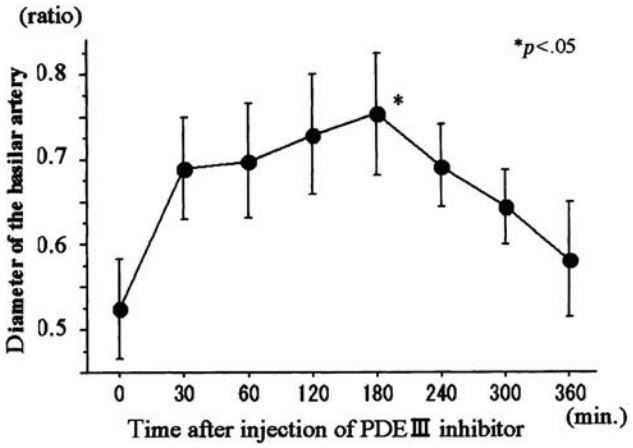


FIGURE 38–2 The effect of intra-arterial injection of milrinone, a phosphodiesterase (PDE) III inhibitor, into the vertebral artery of dogs 7 days after subarachnoid hemorrhage (SAH). Diameter of the basilar artery is expressed as a ratio of the diameter on day 7 to that on day 0. Injection of milrinone resulted in significant dilation of the basilar artery 180 minutes later ($p < .05$). The effect was less marked than that occurring after intracisternal injection. The basilar artery dilated to 75% at most.

Results

Effect of Intracisternal and Intra-arterial Milrinone

The basilar artery on day 7 was contracted to ~60% of its diameter on day 0. Intracisternal injection of milrinone led to significant dilation of the basilar artery dilated after 30 minutes (Fig. 38–1). The effect of milrinone administered by this route decreased with time so that there was significant relaxation of the basilar artery at 30, 60, 120, and 180 minutes after injection. After 360 minutes, the diameter of the basilar artery was not significantly different from its diameter before injection. Intra-arterial injection of milrinone was associated with less vasodilation than intracisternal injection (Fig. 38–2). The basilar artery dilated to some extent between 30 and ~300 minutes after injection but the dilation was significant only 180 minutes after injection, and the magnitude of the dilation was to 75% of normal diameter at most. Almost complete reversal of vasospasm was seen at some times after injection of milrinone intracisternally.

Effects In Vitro of Milrinone

Ring segments of the basilar artery exposed to KCl relaxed in a dose-dependent fashion in response to increasing concentrations of milrinone.

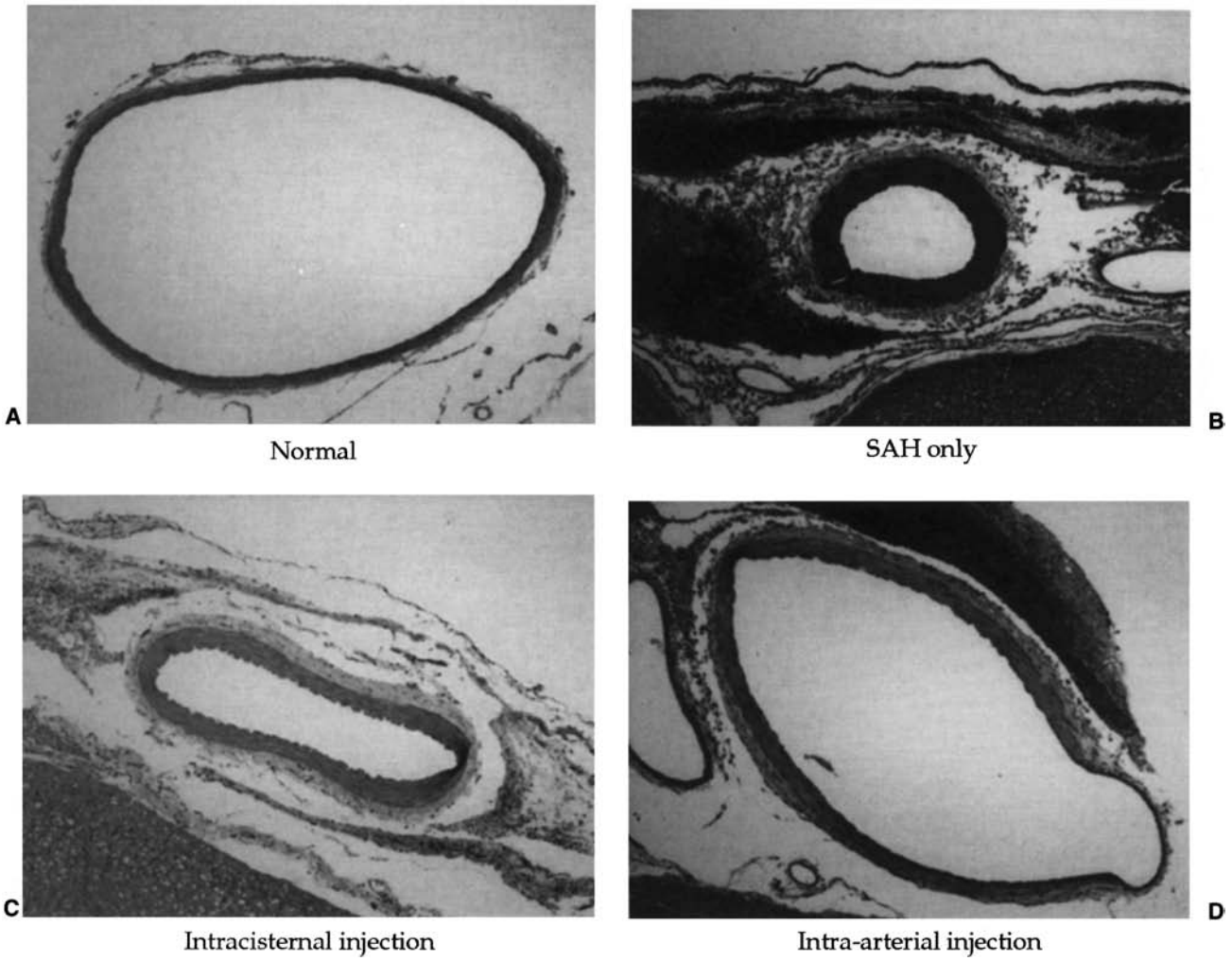


FIGURE 38–3 Photomicrographs of cross sections of dog basilar artery (hematoxylin and eosin, all at original magnification of X 40). (A) A normal dog basilar artery. (B) A basilar artery from a dog with vasospasm 7 days after subarachnoid hemorrhage (SAH). The vessel wall is thick and the vascular lumen is very small. (C) The basilar artery from a dog treated with intracisternal injection of milrinone on day 7 and (D) a dog treated with intra-arterial injection on day 7. After the injection of milrinone, there is relaxation of the smooth muscle cells, with the vessel wall becoming thinner.

Histology and Immunohistochemistry

Histological examination of cross sections of dog basilar artery 7 days after SAH showed that the basilar artery was surrounded by blood clot. The vessel wall was thickened, and the vascular lumen was markedly decreased in caliber (Fig. 38–3). Basilar arteries that had been exposed to intracisternal or intra-arterial milrinone had thinner walls, and the vascular lumens were larger, although not to the extent observed in a normal, control basilar artery. High magnification showed corrugation of the internal elastic lamina and hypertrophy of the smooth muscle cells in vasospastic arteries. On the other hand, relaxation of the smooth muscle cells and less corrugation of the internal elastic lamina were seen after injection of milrinone intracisternally or intra-arterially Immunohistochemistry for cAMP showed marked positive staining in the smooth muscle cells of normal dogs. There was less staining for cAMP in the smooth muscle cells in the chronic vasospasm model. Intracisternal or intra-arterial milrinone therapy increased the amount of cAMP staining compared with untreated SAH dog basilar arteries.

Discussion

There are several isoforms of PDE, although PDE III is abundant in cardiac and cerebrovascular smooth muscle. Milrinone selectively inhibits cAMP-specific PDE III enzyme. This can lead to vasodilation and could contribute to reducing cerebral vasospasm.[4] The present results suggest that such vasodilation can occur after intracisternal injection of milrinone and that the effect is relatively long-lasting compared with intra-arterial injection. The intracisternal route therefore would have potential advantages over intra-arterial and intravenous injection, including longer duration of action and avoidance of side effects such as hypotension. In this study, convulsions and hypotension were not observed, although the dogs were under general anesthesia. Disadvantages of the intracisternal route would include a still relatively short half-life leading to only transient efficacy, and the risks and technical difficulties of giving drugs intracisternally. With regard to intra-arterial injection, blood pressure decreased ~10 to 20% during drug administration. This could lead to potential adverse effects in the patient with vasospasm and might require concomitant use of vasopressor agents. We speculate that intravenous administration would be less effective and more dangerous than intra-arterial injection in that higher doses would be required to achieve the same concentrations in the arteries and, because these would be distributed more widely systemically, there likely would be more hypotension.

Conclusion

Intracisternal injection of milrinone was more effective than intra-arterial injection for reversal of cerebral vasospasm in dogs. The single dose tested in this study led to significant dilation of the basilar artery for up to 180 minutes after injection. Furthermore, this effect was not associated with changes in systemic hemodynamic status. Injection of milrinone intracisternally or intra-arterially increased cAMP levels in the smooth muscle cells of the spastic basilar arteries compared with arteries from dogs with SAH and no drug treatment. This drug was effective for the treatment of experimental vasospasm.

REFERENCES

1. Honerjäger P. Pharmacology of bipyridine phosphodiesterase III inhibitors. Am Heart J 1991;121:1939–1944
2. Arakawa Y, Kikuta K, Hojo M, Goto Y, Ishii A, Yamagata S. Milrinone for the treatment of cerebral vasospasm after subarachnoid hemorrhage: report of seven cases. Neurosurgery 2001;48:723–730
3. Khajavi CI, Ayzman I, Shearer D, et al. Prevention of chronic cerebral vasospasm in dogs with milrinone. Neurosurgery 1997;40:354–363
4. Harris AL, Grant AM, Silver PJ, Evans DB, Alousi AA. Differential vasorelaxant effects of milrinone and amrinone on contractile responses of canine coronary, cerebral, and renal arteries. J Cardiovasc Pharmacol 1989;13:238–244

SECTION VI

Clinical—Doppler and Imaging

39

Intraoperative Microvascular Doppler Sonography for Monitoring Vasospasm and Use of Topical Vasodilators During Intracranial Aneurysm Surgery

ENRICO MARCHESE, M.D., ALESSIO ALBANESE, M.D., FEDERICO DI ROCCO, M.D., LUIGI PENTIMALLI, M.D., ANDREA VIGNATI, M.D., GIULIO MAIRA, M.D.

Abstract

Vasospasm due to manipulation of cerebral vessels during aneurysm surgery may have a negative influence on the immediate clinical outcome of the surgery This study was performed to evaluate the efficacy and reliability of intraoperative microvascular Doppler (IMD) sonography in monitoring acute vasospasm during surgery In addition, the effect of topical vasodilators was assessed during intracranial aneurysm surgery Between November 1998 and December 2002, 121 patients (79 women, 42 men) harboring intracranial aneurysms were operated on. The aneurysm was excluded by clipping in all cases. Doppler evaluation was performed in each patient before clipping, after clipping, and after topical application for 5 minutes of a vasodilatatory substance (sodium nitroprusside or papaverine). The arterial blood pressure was maintained at a constant level during the procedure. In 25 patients IMD revealed an increase in blood flow velocities suggesting mechanical spasm of the examined vessel. The topical application of papaverine in 13 cases and sodium nitroprusside in 12 cases resulted in effective vasodilation as documented in all the cases by reduction of blood flow velocities. No complications associated with the use of these agents were recorded. The use of IMD has several advantages. It confirms the complete exclusion of the aneurysmal sac and the correct positioning of the clip with a preservation of patency of the adjacent vessels and documents the occurrence of mechanical vasospasm. If there is mechanical spasm, the use of a topical vasodilator such as papaverine or sodium nitroprusside is a valuable and safe method to restore blood flow.

During the surgical clipping of an intracranial aneurysm, mechanical arterial vasospasm may result from the manipulation of the cerebral arteries that is necessary during dissection for clip placement. We used intraoperative microvascular Doppler (IMD) to detect the onset of this type of vasospasm and to verify the efficacy of the topical vasodilators papaverine and sodium nitroprusside in reversing this spasm. The correlation of the IMD data with the postoperative clinical condition and angiographic outcome showed that this technique is feasible, safe, and reliable.

Material and Methods

Between November 1998 and December 2002, 121 patients harboring 127 aneurysms were operated on. IMD was utilized in all cases. Patients ranged from Hunt and Hess grade 0 to 5 at the time of surgery.[1] Aneurysms were located on the anterior and posterior circulation, and all were excluded by clip placement. In all patients general anesthesia was obtained by means of monitored ventilation, propofol, remifentanil, and muscle relaxant. The pCO_2 was maintained between 28 and 37 mmHg, and systemic blood pressure was kept between 65 and 110 mmHg. Because alterations in these parameters could influence cerebral blood flow velocity, we maintained them at constant values during the operation. This ensured that IMD data were reliable. IMD monitoring was performed using a 20-Mhz microprobe of 1 mm diameter inserted in a number 3 suction cannula and secured to its extremity by bone wax. After surgical exposure and preparation of the aneurysm and adjacent vessels, we proceeded to obtain baseline IMD of the aneurysm and the adjacent feeding and branch arteries (Fig. 39–1). IMD study was repeated after the clip placement.

In 25 of 121 patients, vasospasm due to surgical manipulation was evident on the IMD study obtained after the clip placement. When this was detected, sodium nitroprusside (4 mg diluted in 4 mL of normal saline, n = 12 patients) and papaverine (50 mg in 4 mL of normal saline, n = 13 patients) was applied to the arteries for 5 minutes. The drugs were applied by soaking Gelfoam in the drug and then placing it around the vessels. After 5 minutes we repeated the IMD study. Postoperative angiography was obtained in all patients.

Results

IMD revealed vasospasm produced by the surgical manipulation of the vessels during the procedure of the clip placement in 25 patients (21%, Fig. 39–2). Application of sodium nitroprusside or papaverine led to resolution of vasospasm with a return of the blood flow velocity to values close to those obtained in the baseline study (Fig. 39–3). There did not appear to be any difference in the efficacy of these drugs in resolving the acute vasospasm observed by IMD. No clinical symptoms or signs related to the vasospasm were documented in these patients in the postoperative period, and no side effects of nitroprusside or papaverine, such as hypotension or alteration in heart rate were noted.

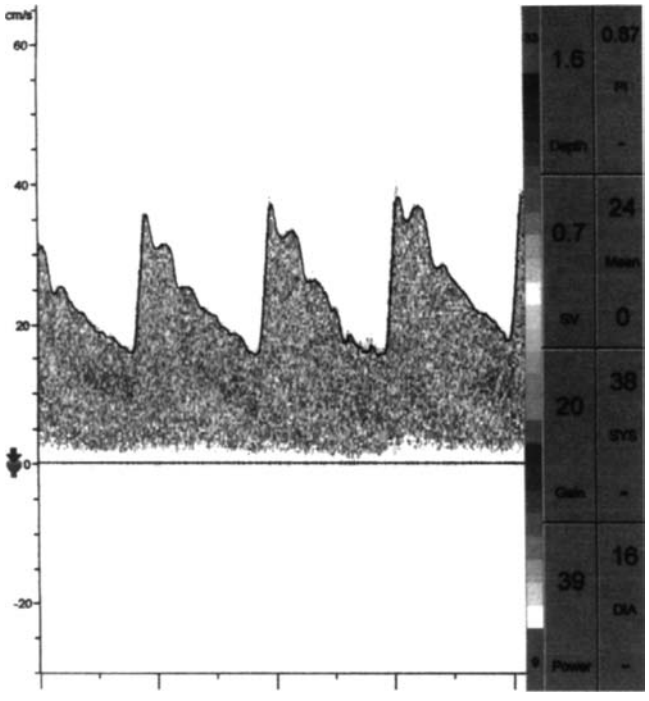


FIGURE 39–1 Baseline intraoperative microvascular Doppler study of the middle cerebral artery before surgical manipulation showing mean flow velocity of 24 cm/sec.

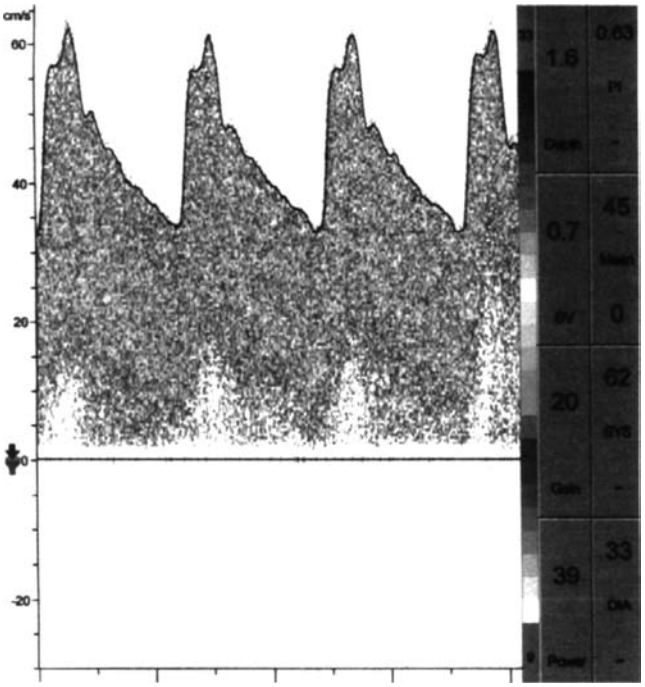


FIGURE 39–2 The middle cerebral artery flow velocity obtained by intraoperative microvascular Doppler after surgical manipulation for clip placement. The mean flow velocity has increased to 45 cm/sec.

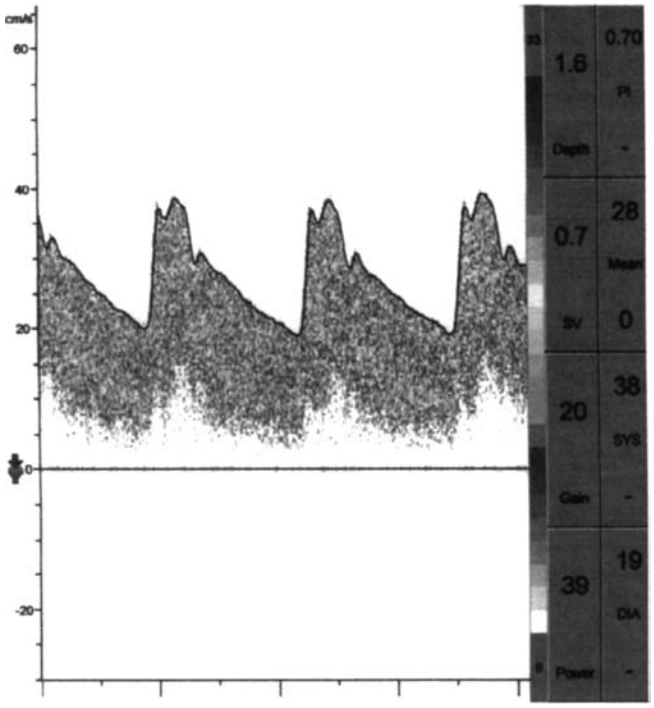


FIGURE 39–3 The intraoperative microvascular Doppler study of the middle cerebral artery 5 minutes after topical application of sodium nitroprusside. The blood flow velocity pattern is close to the baseline study with a mean flow velocity of 28 cm/sec.

Discussion

The first studies regarding the effects of hemorrhage in the subarachnoid space were conducted by Bagley in 1928.[2] In 1949, Jackson described the extraordinary toxicity of subarachnoid deposition of the supernatant fraction of hemolysed blood containing oxyhemoglobin.[3] This produced what he called an "aseptic hemogenic meningitis." One of the first agents utilized to reverse arterial narrowing was papaverine, a potent vasodilator whose activity reverses or blocks the effect of spasmogenic substances present after subarachnoid hemorrhage (SAH).[4] Although we do not know completely the mechanisms upon which cerebral vasospasm is based, recent studies have focused on molecules involved in the control mechanisms of the vascular smooth muscle of the cerebral artery and particularly on the balance between vasoconstrictor and vasodilator factors.[5] Thomas noted that oxyhemoglobin increases endothelin-1 (ET-1) messenger ribonucleic acid in the cerebrospinal fluid, leading to an increase in the level of ET-1, one of the most potent constrictors of mammalian arteries. In addition, oxyhemoglobin inactivates nitric oxide by several mechanisms, including direct binding, thus removing a vasodilator influence and a physiological antagonist of ET-1.[5] Support for this theory was provided by experimental studies demonstrating that, after SAH, oxyhemoglobin produces depletion of nitric oxide in the smooth musculature of the cerebral arteries.[6] Nitric oxide has two relevant physical properties. It is the smallest known biologically active molecule and it has an extremely short half-life. These two characteristics permit this molecule to readily penetrate through the walls of the cerebral arteries and to act in an auto- or paracrine fashion and not decrease the systemic arterial blood pressure.

Thomas and colleagues performed clinical studies based on this hypothesis.[7] They gave intrathecal injections of sodium nitroprusside, a nitric oxide donor, through a ventriculostomy in patients at high risk of vasospasm (Hunt and Hess grades 3–5). Nitroprusside also was given to patients with pharmacologically intractable vasospasm. The study showed that patients with a high Hunt and Hess grade who were treated with nitroprusside did not develop vasospasm and patients with vasospasm who were treated therapeutically showed angiographic resolution of vasospasm in 83% of cases.

Another type of vasospasm is produced by manipulation of the cerebral arteries during aneurysm surgery. The mechanical trauma causes a series of modifications at the level of the cerebrovascular smooth muscle, the most relevant of which is an increase in the permeability of the cell membranes with subsequent intracellular loading of free Ca^{2+} (Ca^{2}+ overloading).[8] The Ca^{2+} is able to activate Ca^{2+}-calmodulin-dependent protein kinase and protein kinase C. Both proteins phosphorylate the contractile proteins of the vascular smooth muscle of the arterial wall, producing smooth muscle contraction.[8] A frequent observation during cerebral aneurysm surgery is spasm of the cerebral arteries after the surgical manipulation necessary to prepare for clip placement. Schaller and Zentner described the postoperative onset of this type of spasm due to mechanical trauma to the arteries in 20 patients who had undergone amygdalohippocampectomy by a transsylvian approach.[9] Transcranial Doppler assessment of the middle cerebral artery in 16 cases documented an increase in flow velocity.

Our protocol for the treatment of mechanical spasm utilized knowledge of the mechanisms leading to vasospasm, namely the balance between vasodilator and vasoconstrictor factors. We preferred topical application to induce a direct action on the vessels of interest. In all cases IMD was utilized to detect the onset of mechanical spasm and to document its

resolution after topical application of sodium nitroprusside or papaverine. In our experience this therapy does not affect systemic blood pressure or heart rate. In the postoperative period no patient showed neurological deficits related to mechanical vasospasm.

Conclusion

IMD permits the noninvasive evaluation of the hemodynamic status of the cerebral arteries during the surgical treatment of aneurysms. Anesthesia, as performed in the preceding text, does not interfere with data obtained by IMD. The cost-effectiveness of IMD is favorable compared with other procedures such as intraoperative angiography. Furthermore, IMD permitted monitoring of the vasospasm produced by surgical manipulation. We consider nitroprusside and papaverine applied topically intraoperatively to be reliable and effective measures to treat this kind of vasospasm.

REFERENCES

1. Hunt WE, Hess RM. Surgical risk as related to time of intervention in the repair of intracranial aneurysms. J Neurosurg 1968;28:14–20
2. Bagley C. Blood in the cerebrospinal fluid: experimental data. Arch Surg 1928;17:18–38
3. Jackson I. Aseptic hemogenic meningitis: an experimental study of aseptic meningeal reactions due to blood and breakdown products. Arch Neurol Psychiatr 1949;62:572–589
4. Kassell NF, Shaffrey ME, Shaffrey CI. Cerebral vasospasm following aneurysmal subarachnoid hemorrhage. In: Apuzzo MLJ, ed. Brain Surgery: Complication Avoidance and Management. New York: Churchill Livingstone; 1992:847–856
5. Thomas JE. Molecular biological considerations in cerebral vasospasm following aneurysmal subarachnoid hemorrhage. Neurosurg Focus 1997;3. Article 3 (on line at website: www.aans.org/education/journal/neurosurgical)
6. Thomas JE, Nemirowski A, Zelman V, Giannotta SL. Rapid reversal of endothelin-1-induced cerebral vasoconstriction by intrathecal administration of nitric oxide donors. Neurosurgery 1997;40:1245–1249
7. Thomas JE, Rosenwasser RH, Armonda RA, Harrop J, Mitchell W, Galaria I. Safety of intrathecal sodium mitroprusside for the treatment and prevention of refractory cerebral vasospasm and ischemia in humans. Stroke 1999;30:1409–1416
8. Towart R. The pathophysiology of cerebral vasospasm and pharmacological approaches to its management. Acta Neurochir (Wien) 1982;63:253–258
9. Schaller C, Zentner J. Vasospastic reactions in response to the transsylvian approach. Surg Neurol 1998;49:170–175

40

Basilar Vasospasm Following Aneurysmal Subarachnoid Hemorrhage: TCD and SPECT Correlation

GILL E. SVIRI, M.D., M.Sc, REINALDO CORREA, M.D., CINDY MAYER, M.D., DAVID H. LEWIS, M.D., DAVID W. NEWELL, M.D.

Abstract

The purpose of the present study was to find a correlation between transcranial Doppler (TCD) flow velocities in the posterior circulation and regional cerebral blood flow using perfusion brain single photon emission computed tomography (SPECT) imaging to establish the value of TCD monitoring of the posterior circulation. A retrospective analysis was made of 108 patients with aneurysmal subarachnoid hemorrhage (SAH) who had daily TCD flow velocity measurements of the vertebrobasilar arteries and serial cerebral blood flow SPECT imaging. Fifty patients (46%) had TCD flow velocities that were consistent with vasospasm of the posterior circulation according to the criteria suggested by Sloan et al.[1] Forty-two patients (39%) had serial SPECT studies showing hypoperfusion in the territories of the posterior circulation [brain stem: 14 (13%); cerebellum: 16 (14.8%); occipital lobes 6 (6%); thalamus 24 (22%)]. Twelve of 14 patients with brain stem hypoperfusion on SPECT were found to have concordant findings of vasospasm in the vertebrobasilar arteries on TCD. Patients with increased TCD flow velocities in the posterior circulation were at higher risk for hypoperfusion of the brain stem when compared with patients with only anterior circulation vasospasm (24% vs 4%). Eight of 16 patients with cerebellum hypoperfusion on SPECT were found to have concordant findings of vasospasm in the vertebrobasilar arteries on TCD. Twenty of 24 patients with thalamic hypoperfusion on SPECT were found to have vasospasm in the vertebrobasilar arteries on TCD. These findings suggest for the first time that vertebrobasilar vasospasm is associated with reduced regional cerebral blood flow to the brain stem as well as to the cerebellum. Patients with vertebrobasilar vasospasm are also at increased risk to develop hypoperfusion of the thalamic area. We suggest that TCD measurement of the posterior circulation should be routinely performed after aneurysmal SAH to identify patients who are at increased risk for delayed ischemia in the posterior circulation territory

Perfusion studies are used commonly for the diagnosis of vasospasm after aneurysmal subarachnoid hemorrhage (SAH) and many studies have shown a correlation between transcranial Doppler (TCD) findings and cerebral tissue perfusion.[2,3] Although vasospasm of the anterior circulation is well established as a clinical entity that should be evaluated, monitored, and treated, vasospasm of the posterior circulation is much ignored, and there is no study that has been done to show whether basilar artery vasospasm is associated with perfusion impairment to the brain stem. Sloan et al[1] have suggested criteria for the diagnosis of vasospasm in the posterior circulation using TCD. They noted that patients with basilar artery flow velocities higher then 85 cm/sec as measured by TCD had a higher incidence of vasospasm. Increased blood flow velocities, however, may not necessarily be associated with narrowing of the vessel and may imply hyperemic flow.[4–6]

This study examined the relationship between TCD flow velocities in the basilar artery and regional cerebral blood flow (rCBF) to identify the value of TCD monitoring of the posterior circulation and the concordance of elevated basilar artery flow velocities with rCBF impairment to the brain stem.

Materials and Methods

The records of 217 patients with aneurysmal SAH who were admitted to Harborview Medical Center between July 2001 and July 2002 were evaluated. A subgroup of 104 patients (age: 51 ± 14; range: 21–73 years; male: 39, female: 65; Fisher grade[7]: 2.9 ±1.1; Hunt and Hess grade[8]: 2.5 ± 1.2) had a baseline TCD and single photon emission computed tomographic (SPECT) study within the first 48 to 72 hours after the hemorrhage and subsequently had at least one more SPECT study. All patients had daily TCD measurements for the first week following the hemorrhage. Middle and anterior cerebral arteries were insonated through the temporal acoustic window. The vertebral and basilar artery flow velocities were measured though the foramen magnum according to the technique described by Fujioka and Douville.[9] Basilar artery vasospasm was defined as flow velocities > 85 cm/sec.

All patients underwent their first brain Tc-99m SPECT scans by the second postoperative day, which was within 48 to 72 hours of SAH. All images were reconstructed as slices 6 mm thick in the transaxial, coronal, and sagittal planes. Additional brain SPECT images were obtained at subsequent times if the patient's clinical condition warranted.

Results

SPECT imaging showed areas of reduced rCBF occurring in a delayed fashion after SAH that were consistent with vasospasm in 68 patients (65%). Fourteen patients had reduced rCBF in the brain stem (14%), 16 in the cerebellum (15%), 21 in the thalamic nuclei (20%), and 6 in the posterior cerebral artery territory (6%). Fifty patients had basilar artery flow velocities that were consistent with vasospasm. Concordance between reduced rCBF and TCD findings of basilar artery vasospasm was found in 12 of 14 patients who had reduced rCBF in the brain stem, 12 of 16 patients in the cerebellum, 17 of 24 in the thalamic nuclei, and 6 of 6 in the posterior cerebral artery territory (Fig. 40–1).

Ten of 19 (53%) patients with basilar artery flow velocities > 120 cm/sec had SPECT scans that showed reduction in the rCBF to the brain stem, whereas only two of 31 (7%) patients with basilar artery flow velocities between 85 cm/sec and 120 cm/sec and 2 of 54 (4%) patients with basilar artery flow velocities <80 cm/sec had SPECT scan that showed reduction in the rCBF to the brain stem (Fig. 40–2).

Discussion

Although some authors have suggested that increased basilar artery flow velocities after SAH are associated with a poorer outcome,[10,11] the hemodynamic and clinical significance of basilar artery vasospasm remains somewhat unclear. Our findings suggest that there is delayed reduction in brain stem perfusion after SAH that can be associated with vasospasm and

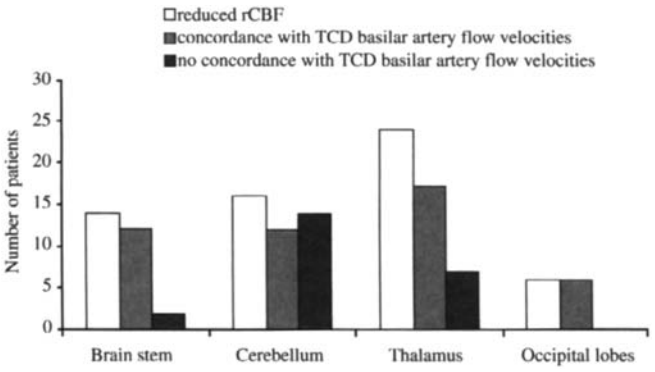


FIGURE 40–1 Concordance between Tc-99m single photon emission computed tomography findings of delayed, reduced perfusion, and transcranial Doppler measurement of increased basilar artery flow velocities (> 85 cm/sec were regarded as demonstrating vasospasm) for different brain territories.

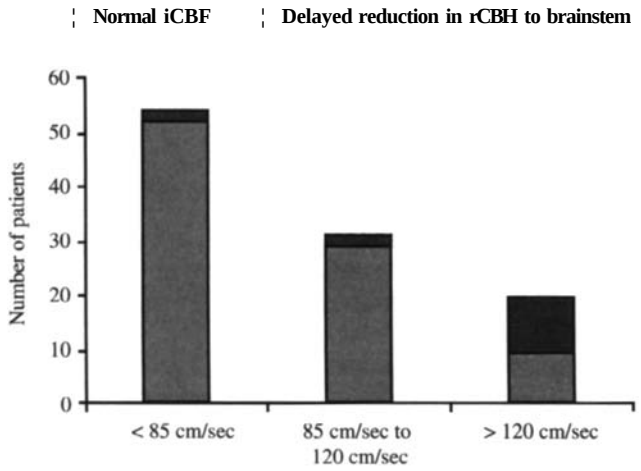


FIGURE 40–2 Proportion of patients with delayed reduction of brain stem regional cerebral blood flow at each of three different values of basilar artery flow velocities (< 85, 85–120, and > 120 cm/sec).

that patients with elevated basilar artery flow velocities measured by TCD are at increased risk to develop this phenomenon. Furthermore, patients with increased basilar artery flow velocities are at increased risk for rCBF disturbance in the thalamic nuclei as well as the cerebellum.

Sloan et al[1] have suggested that the likelihood of basilar artery vasospasm is high when the TCD flow velocity in the basilar artery exceeds 85 cm/sec. However, elevated flow velocities do not differentiate between arterial narrowing and hyperemic flow.[5,6,9] Increased narrowing of the artery may not only reduce the end artery perfusion but also the flow in the perforating arteries. Soustiel and colleagues reported that in a phantom model of the vasculature studied in vitro, significant narrowing of the parent vessel was associated with significantly impaired flow in the perforating arteries arising from the larger parent artery.[12] This is consistent with the finding that patients with very elevated basilar artery flow velocities (> 120 cm/sec) are at higher risk to develop brain stem perfusion defects, which may suggest that those patients suffered from more significant narrowing of the basilar artery in a way that flow to the perforating arteries was impaired.

Conclusion

Increased basilar artery flow velocities can be associated with reduced rCBF to the brain stem secondary to vasospasm. Patients with increased basilar artery flow velocities are at increased risk to have rCBF impairment in the thalamic nuclei as well as the cerebellum. We suggest that patients with SAH should have routine monitoring of basilar artery flow velocities to identify patients who are at increased risk to develop posterior circulation territory perfusion impairments secondary to vasospasm.

REFERENCES

1. Sloan MA, Burch CM, Wozniak MA, et al. Transcranial Doppler detection of vertebrobasilar vasospasm following subarachnoid hemorrhage. Stroke 1994;25:2187–2197
2. Rajendran JG, Lewis DH, Newell DW, et al. Brain SPECT used to evaluate vasospasm after subarachnoid hemorrhage: correlation with angiography and transcranial Doppler. Clin Nucl Med 2001;26:125–130
3. Leclerc X, Fichten A, Gauvrit JY, et al. Symptomatic vasospasm after subarachnoid haemorrhage: assessment of brain damage by diffusion and perfusion-weighted MRI and single-photon emission computed tomography. Neuroradiology 2002;44: 610–616
4. Lindegaard KF, Nornes H, Bakke SJ, et al. Cerebral vasospasm after subarachnoid hemorrhage investigated by means of transcranial Doppler ultrasound. Acta Neurochir Suppl (Wien) 1998;42:81–84
5. Laumer R, Steinmeier R, Gonner F, et al. Cerebral hemodynamics in subarachnoid hemorrhage evaluated by transcranial Doppler sonography, I: Reliability of flow velocities in clinical management. Neurosurgery 1993;33:1–7
6. Romner B, Bellner J, Kongstad P, et al. Elevated transcranial Doppler flow velocities after severe head injury: cerebral vasospasm or hyperemia? J Neurosurg 1996;85:90–97
7. Fisher CM, Kistler JP, Davis JM. Relation of cerebral vasospasm to subarachnoid hemorrhage visualized by computed tomographic scanning. Neurosurgery 1980;6:1–9
8. Hunt WE, Hess RM. Surgical risk as related to time of intervention in the repair of intracranial aneurysms. J Neurosurg 1968;28:14–20
9. Fujioka KA, Douville CM. Anatomy and freehand examination. In: Newell DW, Aaslid R, eds. Transcranial Doppler. New York: Raven; 1992:9–32
10. Soustiel JF, Shik V, Feinsod M. Basilar vasospasm following spontaneous and traumatic subarachnoid haemorrhage: clinical implications. Acta Neurochir (Wien) 2002;144:137–144
11. Lee JH, Martin NA, Alsina G, et al. Hemodynamically significant cerebral vasospasm and outcome after head injury: a prospective study. J Neurosurg 1997;87:221–233
12. Soustiel JF, Levy E, Bibi R, et al. Hemodynamic consequences of cerebral vasospasm on perforating arteries: a phantom model study. Stroke 2001;32:629–635

41

Comparison of Positron Emission Tomography Cerebral Perfusion with Transcranial Doppler in Subarachnoid Hemorrhage Patients with Neurological Deterioration

PAWAN S. MINHAS, M.D., DAVID K. MENON, M.B.B.S., M.D., PH.D., F.R.C.P., F.R.C.A., F.MED.SCI., PIOTR SMIELEWSKI, PH.D., MAREK CZOSNYKA, PH.D., PETER J. KIRKPATRICK, M.B., CH.B., F.R.C.S. (S.N.), JOHN C. CLARK, PH.D., JOHN D. PICKARD, M.D.

Abstract

Transcranial Doppler (TCD) is commonly used as a noninvasive test to detect arterial vasospasm. We have validated TCD against cerebral blood flow measured by positron emission tomography (PET) in 25 patients who developed clinical signs of a delayed neurological deficit following aneurysmal subarachnoid hemorrhage (SAH). The study was approved by the institutional ethics committee. The patients were studied in the Wolfson Brain Imaging Centre and Neurosciences Critical Care Unit if they developed a delayed or global neurological deficit. All patients underwent PET cerebral blood flow and TCD measurements of mean flow velocity in the middle cerebral artery and calculation of the ratio of flow velocity in the middle cerebral to that in the internal carotid artery Glasgow outcome score was assessed at 6 months. Remarkably heterogeneous patterns of cerebral blood flow distribution were observed with hyperemia, normal values, and reduced flow present in patients with delayed neurological deficits. TCD indices (mean flow velocities and the ratio of middle to internal carotid artery flow velocities) were not indicative of cerebral perfusion findings. Mean cerebral blood flow values were slightly lower in patients who did not survive (32 mL/100 g/min) than in those who did survive (36 mL/100 g/min, $p = .05$). Patients developing delayed neurological deficits after aneurysmal SAH displayed a wide range of cerebral perfusion disturbances that were not reflected by the commonly used TCD indices.

Up to 30% of patients suffer a delayed neurological deficit following subarachnoid hemorrhage (SAH). These deficits may be related in part to arterial vasospasm and dysautoregulation. Traditionally, transcranial Doppler (TCD) ultrasound has been used to monitor arterial vasospasm noninvasively TCD has advantages over more invasive tests such as catheter-based angiography, which carries risks of complications.[1] However, there is increasing evidence that the conventional measurements of mean flow velocity of

the middle cerebral artery and the ratio of the flow velocity in the middle cerebral artery to that in the extracranial internal carotid artery (Lindegaard ratio)[2] do not necessarily reflect the evolution of cerebral arterial narrowing, and there is a capricious relationship to the development of delayed cerebral ischemia.[3] However, when TCD is used to detect cerebral dysautoregulation using a transient hyperemic response test, there is a relationship to delayed cerebral ischemia and outcome.[3] To explore this paradox, we have compared cerebral perfusion patterns determined by positron emission tomography (PET) with TCD indices in 25 patients with aneurysmal SAH who developed clinical signs of delayed neurological deficit.

Patients and Methods

Twenty-five patients [15 females and 10 males with a mean age of 55 years (range 25–74)] with aneurysmal subarachnoid hemorrhage (SAH) confirmed by digital subtraction angiography were studied within the environment of the Neurosurgical Intensive Care Unit and the Wolfson Brain Imaging Center. Local research ethics committee approval was obtained for the study Conventional therapy included nimodipine and hypertensive, hypervolemic, hemodilutional therapy with a target mean arterial pressure of 100 to 120 mmHg. Patients were studied if they developed a new neurological deficit, either global with reduction in the Glasgow coma score or focal with development of dysphasia or motor limb weakness, and where secondary insults such as hyponatremia, hypoxia, hyperpyrexia, seizures, or hydrocephalus had been excluded by appropriate laboratory and radiological testing. TCD flow velocities (Neuroguard TCD system, Medsonics, Freemont, CA, USA) were recorded at the time of PET scanning. The middle cerebral artery flow velocities from both hemispheres were recorded together with the Lindegaard ratios (ratio of flow velocity in the middle cerebral artery to that in the ipsilateral extracranial internal carotid artery). A Lindegaard ratio of more than 3 is said to suggest vasospasm. Presenting World Federation of Neurological Surgeons grades[4] were grade 1 (n = 1), 2 (n = 7), 3 (n = 9), 4 (n = 5), and 5 (n = 3). Median day of study was 6 days following SAH (range 1–13 days). A GE Advance PET scanner was used together with the steady state $H_2{}^{15}O$ cerebral blood flow technique (see reference[5] for full details). A standardized, three-dimensional middle cerebral artery territory region of interest was applied to the normalized PET emission data for calculation of regional cerebral blood flow in the middle cerebral artery territory. A middle cerebral artery region of interest cerebral blood flow of < 30 m.L/100 g/min was considered ischemic whereas a value of > 50 mL/100 g/min was considered hyperemic.

Results

Fifteen patients presented with either hemiparesis or dysphasia of whom only five demonstrated elevated flow velocities and Lindegaard ratios of more than 3. For these five patients, the TCD findings were consistent with the side of the neurological deficit. PET cerebral blood flow, however, demonstrated appropriate middle cerebral artery ischemia in two patients, hyperemia in one, and no abnormality in two. In the 10 patients with normal TCD findings, normal PET perfusion was seen in five patients, appropriate ischemia in four, and mild hyperemia in one patient. Interestingly, there was no relationship between TCD flow velocity and PET cerebral blood flow. Elevated or normal flow velocities were not predictive of ischemia, hyperemia, or normal cerebral blood flow. There was no significant correlation between the Lindegaard ratio and PET cerebral blood flow.

Discussion

PET cerebral blood flow measurements revealed a wide variation in cerebral blood flow pattern ranging from reduced flow to normal cerebral blood flow values to hyperemia among patients with delayed neurological deterioration after aneurysmal SAH. Neither mean flow velocity nor the Lindegaard ratio was helpful in predicting the cerebral blood flow patterns observed on PET. Our results support the growing awareness that large vessel vasospasm is but one component of a more complex sequence of events leading to delayed neurological deficit. Ideally, serial studies of both cerebral blood flow and cerebral metabolism are required to more accurately define the sequence of events leading to the development of delayed neurological deficits and to determine the factors that contribute to reversibility or irreversibility of such deficits. TCD may continue to have a useful role in the assessment of patients with SAH provided that it is used to interrogate the status of autoregulation using, for example, the transient hyperemia response test.[3] Other developments such as the use of the spectral intensity-weighted waveform analysis may provide an index of actual blood flow volume through the vessel from which a TCD flow velocity is obtained.

In conclusion, caution should be exercised when using TCD flow velocities and Lindegaard ratios for guiding therapy for patients with delayed neurological deficits after aneurysmal SAH. Regional cerebral blood flow should ideally be assessed rapidly prior to institution of therapies that may carry significant risk, such as aggressive hemodynamic manipulations.

Acknowledgment

Supported by a Medical Research Council Program Grant for the Study of Acute Brain Injury, a Clinical Research Training Fellowship to Pawan Minhas from the Royal College of Surgeons of England and the Medical Research Council (UK).

REFERENCES

1. Aaslid R, Huber P, Nornes H. Evaluation of cerebrovascular spasm with transcranial Doppler ultrasound. J Neurosurg 1984;60:37–41

2. Lindegaard KF, Nornes H, Bakke SJ, Sorteberg W, Nakstad P. Cerebral vasospasm after subarachnoid haemorrhage investigated by means of transcranial Doppler. Acta Neurochir Suppl (Wien) 1988;42:81–84

3. Lam JMK, Smielewski P, Czosnyka M, Pickard JD, Kirkpatrick PJ. Predicting delayed ischaemic deficits after aneurysmal subarachnoid haemorrhage using a transient hyperaemic response test of cerebral autoregulation. Neurosurgery 2000;47:819–826

4. Drake CG, Hunt WE, Sano K, et al. Report of World Federation of Neurological Surgeons Committee on a Universal Subarachnoid Hemorrhage Grading Scale. J Neurosurg 1988;68:985–986

5. Minhas PS, Menon DK, Smielewski P, et al. Positron emission tomographic cerbral perfusion disturbances and transcranial Doppler findings among patients with neurological deterioration after subarachnoid hemorrhage. Neurosugery 2003;52:1017–1024

42

Brain Perfusion Computed Tomography in Severe Symptomatic Vasospasm

GILL E. SVIRI, M.D., M.Sc, WENDY COHEN, M.D., REINALDO CORREA, M.D., SUDAKAR PIPAVATH, M.D., DAVID W. NEWELL, M.D.

Abstract

Dynamic computed tomographic (CT) perfusion has become a widely accepted imaging modality for diagnostic workup of acute stroke patients. However, this method has not yet been used for diagnosis of delayed ischemia after subarachnoid hemorrhage (SAH). We present preliminary findings using the perfusion CT scan for diagnosis of cerebral ischemia in patients with severe symptomatic vasospasm. Fifteen patients with severe clinically symptomatic vasospasm following aneurysmal SAH who had reduced regional cerebral blood flow associated with vasospasm on single photon emission CT (SPECT) and/or moderate to severe vasospasm on transcranial Doppler (TCD) ultrasound measurement had perfusion CT scans of the head. Multiparameter imaging using maps of cerebral blood flow, cerebral blood volume, and the time parameter of the local bolus transit was done to enable detailed analysis of cerebral perfusion status. We looked for concordance with other imaging modalities (angiography, SPECT, and TCD). Twelve of 15 patients had perfusion CT findings that were associated with delayed ischemia. Perfusion CT was found to have a high concordance with other diagnostic modalities (in 11 of 15 patients we found a good correlation with angiography, 13 of 15 patients agreed with TCD, and 12 out of 15 patients were concordant with SPECT). In three patients the perfusion CT scan was found to be more sensitive than SPECT. In all patients the local bolus mean transit time was found to be more sensitive for detection of involved brain territories than either or both cerebral blood flow and cerebral blood volume. Preliminary results show that perfusion CT can be a sensitive modality for the diagnosis of ischemic brain territories in patients with severe cerebral vasospasm. Perfusion CT scan has the advantages of being quick, repeatable, and easier to perform than SPECT, and the test can be done at any time point on a spiral CT. Further study should be done to evaluate the role of perfusion CT for the assessment of delayed ischemia in patients with SAH and to compare it with other imaging modalities.

Dynamic computed tomographic (CT) perfusion is a CT-based imaging procedure that provides quantitative maps of cerebral blood flow (CBF), cerebral blood volume (CBV), and local mean bolus transit time (MTT).[1,2] This allows detailed analysis of cerebral perfusion status. The dynamic CT perfusion technique has the advantage of being readily available and accessible in the emergency setting. It has become a part of the imaging armamentarium in the diagnostic workup of patients with acute stroke.[3] However, this method has not yet been evaluated for diagnosis of delayed ischemia following subarachnoid hemorrhage (SAH). We present preliminary findings using the perfusion CT scan for diagnosis of cerebral ischemia in patients with severe symptomatic cerebral vasospasm.

Patients and Methods

Fifteen patients with symptomatic cerebral vasospasm following aneurysmal SAH had 23 dynamic CT perfusion scans. All patients had transcranial Doppler (TCD) measurements and /or Tc-99m hexamethyl propyleneamine oxime and ethyl cysteine dimer perfusion brain single photon computed tomographic scan (SPECT) imaging done within 12 hours of the dynamic CT perfusion scan. TCD was performed and vasospasm severity was graded according to criteria suggested by Aaslid et al[4] and Lindegaard et al.[5] All patients had TCD findings of severe vasospasm or reduced cerebral blood flow or both on SPECT scan and these changes were attributed to vasospasm. Fifteen patients had 17 arteriograms done within 12 hours of the dynamic CT perfusion study. All patients were treated in a neurosurgical intensive care unit and received nimodipine and hypertensive, hypervolemic, hemodilutional therapy guided by the use of pulmonary arterial catheters. In 14 patients the target mean arterial pressure was 110 mmHg, and the wedge pressure was 14 to 16 cm H_2O. All patients had the ruptured aneurysm treated by surgical clipping or endovascular treatment with Guglielmi detachable coils within 72 hours of SAH.

The dynamic CT perfusion studies were performed using an eight-slice helical CT scanner (GE Medical systems, Milwaukee, WI, USA). Two levels were typically scanned: the basal ganglia and the top of the lateral ventricles. Scans were obtained every second for 55 seconds during bolus infusion of 45 mL of iodixanol (Visipaque™, Amersham, Arlington Heights, IL, USA) at 4 mL/sec. Data were transferred to an offline workstation for calculation of cerebral blood flow maps using commercially available software. Calculations were based upon the central volume principle, which has been described elsewhere.[1,6–8] CBF, CBV, and MTT were displayed as color maps. Numerical data could be extracted from the maps. Normal mixed cortical CBF was 50 mL/100 gm/min, normal CBV was 1 to 2 mL/100 g, and normal MTT was 2 to 4 seconds.

Results

Areas of reduced perfusion were found in 16 of 23 dynamic CT perfusion scans (70%). MTT was elevated in 20 of 23 scans (88%), and CBV was reduced in only 10 cases (44%, Fig. 42–1). Twelve dynamic CT perfusion scans showed areas of hyperemic flow. TCD measurements were done within 12 hours of the dynamic CT perfusion scans in 19 of 23 cases. Severe vasospasm was found in 24 of 35 middle cerebral arteries that were measured. Seventeen of these were in concordance with dynamic CT perfusion scans (71%). Vasospasm of the anterior cerebral artery was found in 26 vessels. Concordance with dynamic CT perfusion was found in 19 cases (73%, Fig. 42–2). In 12 patients, however, dynamic CT perfusion scans demonstrated areas of hyperemic flow.

Fifteen patients had 18 SPECT studies that were associated with vasospasm. In 14 (78%) cases the dynamic CT perfusion scans showed reduced CBF and in 15 (83%), scans revealed increased MTT. However, only in 13 (72%) of these cases was there a territorial concordance (see Fig. 42–2). In seven dynamic CT perfusion scans there were brain areas that had hyperemic flow that appeared as normal CBF on SPECT. Furthermore, dynamic CT perfusion was more sensitive to perfusion changes around hematomas or areas that were already infarcted.

Seven patients had 11 dynamic CT perfusion scans that showed areas of increased MTT of > 7 seconds

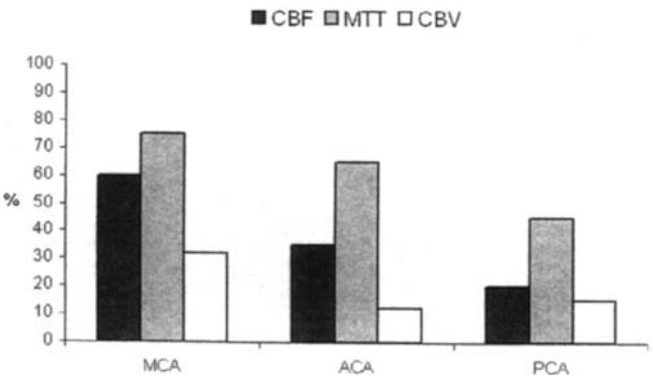


FIGURE 42–1 Percent of patients (n = 23 studies) who were found to have abnormalities in cerebral blood flow (CBF), mean transit time (MTT), and cerebral blood volume (CBV) in the middle cerebral artery (MCA), anterior cerebral artery (ACA), and posterior cerebral artery (PCA) territories on 23 dynamic computed tomographic perfusion scans done on 15 patients with symptomatic cerebral vasospasm.

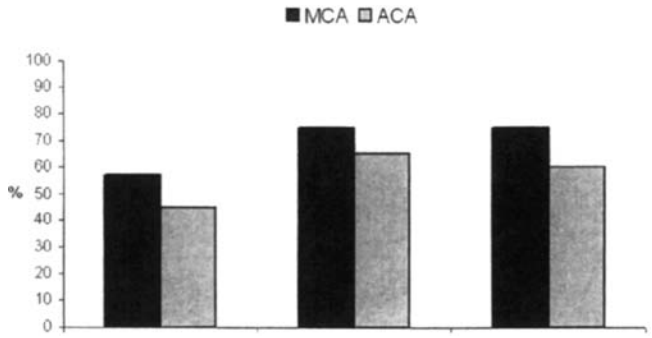


FIGURE 42–2 Concordance between the dynamic computed tomographic perfusion scans and transcranial Doppler (TCD), single photon positron emission tomography imaging, and angiography TCD bars are percent concordance with severe vasospasm for middle cerebral (MCA) and anterior cerebral arteries (ACA). Angiography bars are concordance with severe vasospasm for the same arteries.

and/or perfusion of < 30 mL/100 g/min. In five of the patients the changes progressed to infarction. Seventeen cerebral angiograms were done within 12 hours of the CT perfusion scan. Sixteen middle cerebral arteries were found to have severe narrowing on angiography. The CBF was decreased and/or there was increased MTT in 12 of these cases (75%, see Fig. 42–2).

Discussion

Dynamic CT perfusion appears to be a sensitive test for diagnosis of impaired cerebral hemodynamics in patients with cerebral vasospasm after aneurysmal SAH. Brain territories with increased MTT or decreased CBF were found in 88% of patients who had delayed neurological deterioration related to vasospasm. MTT was found to be more sensitive for flow changes than CBF. CBV was found to be the least sensitive. The concordance of vasospasm with TCD findings was high, although this was mainly in cases where the patient had severe vasospasm of the middle cerebral artery by TCD. There was a group of patients who had areas of hyperemia demonstrated by dynamic CT perfusion at the same time that TCD measurements suggested cerebral vasospasm. We believe that these hyperemic areas can be explained by loss of autoregulation and/or compensatory flow changes. These physiological changes can and may be augmented by the hypertensive, hypervolemic, and hemodilution therapy that is used in these patients.

Concordance of dynamic CT perfusion with SPECT was high (77%) and furthermore, dynamic CT perfusion scanning was found to be predictive of the development of cerebral infarction. Patients who had areas of reduced perfusion of < 30 mL/100 g/min or elevated MTT > 7 seconds or both had a higher incidence of cerebral infarction.

Dynamic CT perfusion has the major advantage of being able to assess CBF, CBV, and MTT in a quantitative way allowing direct insight into cerebral vascular autoregulation.[3] The salvageable brain territories in patients with cerebral vasospasm can potentially be identified. The study is easily obtained in the emergency setting and typically is performed in association with a conventional cranial CT examination. This avoids transfer to other imaging areas and can expedite decision making and therapy in the unstable, deteriorating patient with cerebral vasospasm. Critically ill patients can be examined without compromise of life-support equipment (such as might be required with perfusion magnetic resonance imaging or SPECT) due to the rapid scan time. Results are quantitative, allowing comparison to prior studies as well as to established norms. The technological requirements are limited to software on a standard CT scanner as well as the post-processing software on a CT workstation.[8]

Conclusion

Dynamic CT perfusion can be a valuable technique to evaluate cerebral hemodynamic status including CBF in patients with symptomatic vasospasm. Although the preliminary results presented here are favorable and of interest, further work is required to define the sensitivity and specificity of this examination as well as its predictive value in patients with aneurysmal SAH and cerebral vasospasm.

REFERENCES

1. Wintermark M, Maeder P, Thiran JP, et al. Simultaneous measurements of regional cerebral blood flow by perfusion-CT and stable xenon-CT: a validation study. AJNR Am J Neuroradiol 2001;22:905–914
2. Gillard JH, Antoun NM, Burnet NG, et al. Reproducibility of quantitative CT perfusion imaging. Br J Radiol 2001;74:552–555
3. Wintermark M, Bogousslavsky J. Imaging of acute ischemic brain injury: the return of computed tomography. Curr Opin Neurol 2003;16:59–63
4. Aaslid R, Huber P, Nornes H. Evaluation of cerebrovascular spasm with transcranial Doppler ultrasound. J Neurosurg 1984;60:37–41
5. Lindegaard KF, Nornes H, Bakke SJ, et al. Cerebral vasospasm after subarachnoid hemorrhage investigated by means of transcranial Doppler ultrasound. Acta Neurochir Suppl (Wien) 1998;42:81–84
6. Wintermark M, Reichhart M, Maeder P, et al. Comparison of admission perfusion computed tomography and qualitative diffusion- and perfusion-weighted magnetic resonance imaging in acute stroke patients. Stroke 2002;33:2025–2031
7. Wintermark M, Reichhart M, Thiran JP, et al. Prognostic accuracy of cerebral blood flow measurement by perfusion computed tomography, at the time of emergency room admission, in acute stroke patients. Ann Neurol 2002;51:417–432
8. Eastwood JD, Lev MH, Azhari T, et al. CT perfusion scanning with deconvolution analysis: pilot study in patients with acute middle cerebral artery stroke. Radiology 2002;222:227–236

43

Vasospasm and Regional Brain Perfusion: Correlation Between TCD and CT Perfusion Measurement

HEIKO MEWES, M.D., MATTHIAS F. OERTEL, M.D., MONIKA HÜGENS-PENZEL, M.D., DIETER-KARSTEN BÖKER, M.D., WOLFGANG DEINSBERGER, M.D.

Abstract

Transcranial Doppler ultrasound allows quantification of flow velocities in larger cerebral arteries and therefore can provide information about the diameter of these arteries assuming relatively stable cerebral blood flow. Cerebral blood flow, however, may be markedly altered after subarachnoid hemorrhage, making interpretation of flow velocities difficult. Computed tomographic (CT) perfusion, an indicator-dilution method using a nondiffusible marker, determines regional mean transit time and blood volume. Regional cerebral blood flow can be calculated from these values. This method enables fairly simple determination of cerebral blood flow. This study examined 17 patients with spontaneous SAH with CT pefusion. Forty mL of a nonionic contrast medium with a concentration of 300 mg/mL was injected within 10 seconds via a cubital vein or central venous catheter. After an interval of 0 to 8 seconds (depending on the position of the venous catheter), 45 layers (scan time 1 sec) were scanned. The calculation of parameters was done at an independent workstation with the slow-injection and deconvolution method. All patients underwent daily transcranial Doppler ultrasound and the flow velocities were correlated with the results of the CT perfusion measurements. There was no correlation between transcranial Doppler flow velocities and CT perfusion blood flow. The clinical course often corresponded more closely with the findings on CT perfusion. Early changes on the perfusion measurements corresponded well with the clinical course and findings on the native CT scans. It is concluded that the CT perfusion method is useful for measurement of regional brain perfusion. The method can give important information in patients who show clinical changes in the absence of substantial abnormalities on other diagnostic tests such as transcranial Doppler ultrasound.

Delayed ischemic neurological deficit caused by cerebral vasospasm is an important factor determining the outcome of patients after subarachnoid hemorrhage (SAH). Therefore, measurement of brain perfusion may be clinically useful to identify patients at risk of cerebral ischemia. Transcranial Doppler (TCD) ultrasound has been commonly employed as a method to assess cerebral perfusion. It allows the quantification

of flow velocities and therefore provides some information about the diameter of the great basal brain vessels. Cerebral blood flow (CBF) has been measured by methods such as single photon emission computed tomography (SPECT) and xenon computed tomography (CT). An emerging technology for rapid, simple assessment of CBF is CT perfusion.[1–3] The purpose of this study was to test the hypothesis that the new method of CT perfusion is able to detect cerebral ischemia after SAH.

Patients and Methods

Seventeen patients with aneurysmal SAH were included in the study. The male to female ratio was 1:1.5. The mean age was 55 ± 20 years. Three patients were admitted as Hunt and Hess grade 1, seven as grade 2, four as grade 3, and three as grade 4.[4] Angiography revealed 18 aneurysms in 17 patients (4 at the anterior communicating artery complex, 6 at the middle cerebral artery, 5 at the posterior communicating artery, and 2 on the basilar artery). Twelve patients were operated on and five patients were treated endovascularly. Treatment was performed within 36 hours of the ictus.

CT perfusion was performed at three times (1–2, 3–5, and 7–9 days) after SAH. TCD was performed on a daily basis and initially before the CT perfusion measurement. Vasospasm was defined as flow velocities > 120 cm/sec and a Lindegaard ratio > 3.[5] CT perfusion was performed by injection of 40 mL of a nonionic contrast medium with a concentration of 300 mg/mL into a cubital vein or central venous catheter over 10 seconds. After an interval of 0 to 8 seconds, depending on the position of the venous catheter, 45 layers (scan time 1 sec) were scanned. All CT perfusion scans were analyzed on an imaging workstation (Advantage Windows, G.E. Medical Systems, Waukesha, WI, USA) with commercial analysis software (CT PERFUSION, G.E. Medical Systems). Mean transit time and cerebral blood volume were determined by the deconvolution method. Cerebral blood flow was then computed by using the central volume principle that defines CBF as the ratio of cerebral blood volume to mean transit time. Mean values across and over time were compared for patients with vasospasm who developed an infarction, patients with vasospasm but no infarction, and a control group of patients without vasospasm. Comparison of two parameters was by *t*-test, and multiple comparisons was by analysis of variance. Correlation was assessed by Pearson linear correlation. Statistical significance was accepted at the $p < .05$ level.

Results

Thirteen patients developed vasospasm, and 7 of these had cerebral infarction, as determined by cranial CT scan, 9 to 12 days after SAH. Comparison of CT perfusion measurement of the symptomatic with the asymptomatic side in patients with infarction showed significant differences in CBF, mean transit time, and TCD. The difference in cerebral blood volume was not significant but showed a trend toward larger volumes on the symptomatic side (Table 43–1).

In patients with no infarction from vasospasm, there were no significant differences between the hemispheres in TCD, CBF, and mean transit time (Table 43–2). Cerebral blood volume was virtually identical in both hemispheres. TCD flow velocities were significantly increased in patients with infarction and those with no infarction compared with control patients (132, 108, and 74 cm/sec, respectively, $p = .0095$). CBF was decreased in patients with infarction from vasospasm (35 mL/100 g/min) compared with patients with no infarction (43 mL/100 g/min) and to the control group (42 mL/100 g/min, Fig. 43–1). The difference between the infarction and no infarction group was statistically significant ($p = .017$). Mean transit time was significant prolonged in the infarction group (3.6 sec) compared with 3.1 and 2.8 seconds in vasospasm patients without infarction and controls, respectively ($p = .028$ and .00027, respectively). Cerebral blood volume was significantly increased (2.9 mL/100 g) in patients with infarction compared with the no infarction group (2.5 mL/100 g, $p = .019$).

Mean TCD flow velocities increased over time in patients with infarction from 99 cm/sec on days 1 to 2 to 140 cm/sec on days 3 to 5 and 157 cm/sec on days 7 to 9. CBF increased from day 1 to 2 (37 mL/100 g/min)

TABLE 43–1 Comparison of Transcranial Doppler, Cerebral Blood Volume, Cerebral Blood Flow, and Mean Transit Time in Patients with Infarction from Vasospasm ($n = 7$)

Parameter	Symptomatic Side	Asymptomatic Side	*p* Value
Transcranial Doppler Ultrasound (cm/sec)	132 ± 33	88 ±17	.02
Cerebral Blood Volume (mL/100 g)	2.9 ± 0.4	2.5 ± 0.4	.11
Cerebral Blood Flow (mL/100 g/min)	35 ±5	38 ±3	.09
Mean Transit Time (sec)	3.6 ± 0.5	2.9 ± 0.4	.03

TABLE 43–2 Comparison of Transcranial Doppler, Cerebral Blood Volume, Cerebral Blood Flow, and Mean Transit Time in Patients without Infarction from Vasospasm (n = 6)

Parameter	Symptomatic Side	Asymptomatic Side	p Value
Transcranial Doppler Ultrasound (cm/sec)	109 ± 25	73 ±10	**.03**
Cerebral Blood Volume (mL/100 g)	2.6 ± 0.2	2.6 ± 0.3	**.96**
Cerebral Blood Flow (mL/100 g/min)	44±5	41 ±2	**.22**
Mean Transit Time (sec)	3.1 ± 0.3	2.8 ± 0.5	**.22**

to day 3 to 5 (41 mL/100 g/min) and significantly decreased on day 7 to 9 (26 mL/100 g/min, p = .00058). Mean transit time was prolonged on days 7 to 9 (3.9 sec) compared with 3.4 seconds on days 1 to 2 and 3.9 seconds on days 3 to 5 (p = .17). Cerebral blood volume was significantly increased on days 3 to 5 (3.3 mL/100 g) and 7 to 9 (3.2 mL/100 g) compared with 2.2 mL/100 g in controls (p = .015). There was no correlation between TCD flow velocities and CBF, cerebral blood volume, and mean transit time (r = -0.39, 0.32, and 0.14 for the three correlations, respectively).

Discussion

CT perfusion is a new method to measure brain perfusion. Nabavi et al studied this method in an animal model and presented preliminary clinical results suggesting that this new method is an accurate and cost-effective technique for measuring blood flow.[6] In another clinical study, Wintermark and colleagues found a good correlation between CBF values obtained with CT perfusion and values obtained with xenon CT in patients with various neurological disorders.[7] CBF in normal brain was 49 ± 25 mL/100 g/min based on CT perfusion and 46 ± 24 mL/100 g/min using xenon CT measurement. Values for CBF and cerebral blood volume obtained by positron emission tomography and CT perfusion were similar in patients with cerebral infarction.[8] A study of 15 patients with SAH had similar results to this study.[9] In patients with infarction caused by vasospasm, CBF was decreased to 34 mL/100 g/min, and cerebral blood volume was increased to 3.6 mL/100 g. These values also are similar to those produced in similar patients using other techniques for measurement such as positron emission tomography and xenon CT examination.

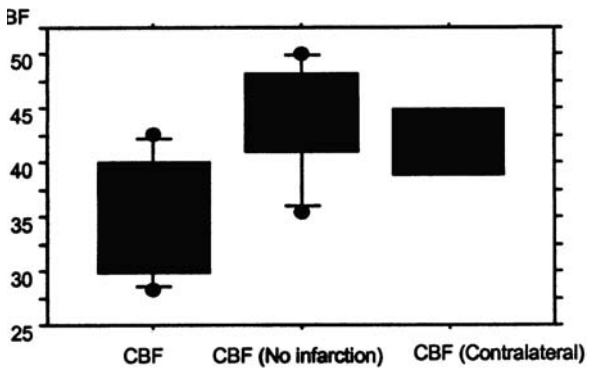


FIGURE 43–1 Cerebral blood flow (CBF) values for patients with cerebral infarction from vasospasm (CBF), no infarction (CBF no infarction), and on the contralateral hemisphere of patients with infarction (CBF contralateral). Boxes are mean values and error bars are standard deviations.

In conclusion, CT perfusion is a new, minimally invasive method to monitor regional brain perfusion. These preliminary results suggest that it provides important information in patients who are at risk for cerebral ischemia and infarction. The addition of CT perfusion to TCD measurements facilitates therapeutic decisions in patients after SAH.

REFERENCES

1. Harders AG, Gilsbach JM. Time course of blood velocity changes related to vasospasm in the circle of Willis measured by transcranial Doppler ultrasound J Neurosurg 1987;66:718–728
2. Cenic A, Nabavi DG, Craen RA, Gelb AW, Lee TY. Dynamic CT measurement of cerebral blood flow: a validation study. AJNR Am J Neuroradiol 1999;20:63–73
3. Eastwood JD, Lev MH, Azhari T, et al. CT perfusion scanning with deconvolution analysis: pilot study in patients with acute middle cerebral artery stroke. Radiology 2002;222:227–236
4. Hunt WE, Hess RM. Surgical risk as related to time of intervention in the repair of intracranial aneurysms. J Neurosurg 1968;28:14–20
5. Lindegaard KF, Nornes H, Bakke SJ, et al. Cerebral vasospasm after subarachnoid hemorrhage investigated by means of transcranial Doppler ultrasound. Acta Neurochir Suppl (Wien) 1998;42:81–84
6. Nabavi DG, Cenic A, Craen RA. CT assessment of cerebral perfusion: experimental validation and initial clinical experience. Radiology 1999;213:141–149
7. Wintermark M, Thiran JP, Maeder P, Schnyder P, Meuli R. Simultaneous measurement of regional cerebral blood flow by perfusion CT and stable xenon CT: a validation study. AJNR Am J Neuroradiol 2001;22:905–914
8. Nakane H, Ibayashi S, Fujii K, et al. Cerebral blood flow and metabolism in patients with silent brain infarction: occult misery perfusion in the cerebral cortex J Neurol Neurosurg Psychiatry 1998;65:317–321
9. Nabavi DG, Le Blanc LM, Baxter B, et al. Monitoring cerebral perfusion after subarachnoid hemorrhage using CT. Neuroradiology 2001;43:7–16

44

Regional Cerebral Blood Flow Monitoring for the Diagnosis of Delayed Ischemia after Aneurysmal Subarachnoid Hemorrhage

PETER VAJKOCZY, M.D., PETER HORN, M.D., CLAUDIUS F.C. THOMÉ, M.D., RLKE MUNCH, M.D., PETER SCHMIEDEK, M.D.

Abstract

Current monitoring of patients with subarachnoid hemorrhage (SAH) usually does not include a continuous assessment of cerebral blood flow (CBF) obtained at the bedside. This study evaluated regional CBF monitoring by thermal diffusion (TD) flowmetry as a novel means for the bedside diagnosis of vasospasm-related cerebral hypoperfusion. Fourteen patients with high-grade SAH who underwent early clipping of anterior circulation aneurysms were prospectively entered into the study. Thermal diffusion microprobes were implanted into the white matter of vascular territories at risk for developing symptomatic vasospasm. Data on arterial blood pressure, intracranial pressure, cerebral perfusion pressure, TD-regional cerebral blood flow (rCBF), cerebrovascular resistance (CVR), and blood flow velocities were collected at the beside. The diagnosis of symptomatic vasospasm was based on the manifestation of either or both a delayed ischemic neurological deficit and a reduced territorial CBF as assessed by stable xenon-computed tomography (CT) in combination with vasospasm demonstrated by angiography. Bedside monitoring of TD-rCBF and CVR allowed detection of symptomatic vasospasm. In the group of patients with vasospasm (n = 10), TD-rCBF decreased from 21 ± 4 to 9 ± 1 mL/100 g/min (mean ± standard error of the mean), whereas TD-rCBF remained unchanged in patients without vasospasm (n = 4; TD-rCBF = 25 ± 4 vs 21 ± 4 mL/100 g/min). Comparing TD-rCBF and xenon-CT results as well as calculating sensitivities, specificities, predictive values, and likelihood ratios, identified a TD-rCBF value of 15 mL/100 g/min as a reliable cutoff for the diagnosis of symptomatic vasospasm. In addition, TD flowmetry was characterized by a more favorable diagnostic reliability than transcranial Doppler sonography. TD flowmetry represents a promising technique for the bedside monitoring of SAH patients to detect symptomatic vasospasm. This is of major clinical interest for high-grade SAH patients that often cannot be assessed neurologically

The diagnosis of symptomatic or hemodynamically relevant vasospasm still remains a problem in the treatment of subarachnoid hemorrhage (SAH) patients. This applies especially to high-grade SAH patients that are comatose or have to remain sedated, and, thus, are not easily assessed by neurological examination. Cerebral angiography remains the gold standard in the diagnosis of cerebral vasospasm. However, the specificity for angiography in the diagnosis of symptomatic vasospasm has been recently calculated to be 50%, which indicates that in cases where the patient cannot be assessed neurologically, the hemodynamic relevance of angiographic vasospasm may be somewhat obscure. Recently, a novel thermal diffusion (TD) microprobe has been introduced for the continuous bedside monitoring of regional cerebral blood flow (rCBF) (TD-rCBF, in mL/100 g/min). Our previous experimental and clinical studies have demonstrated that this technique can reliably detect even discrete rCBF changes.[1–3] Furthermore, the TD-rCBF values have been validated using the stable xenon-enhanced computed tomographic (CT) technique.[3] As a consequence, TD flowmetry may be a promising technique to overcome the obstacles in the reliable detection of symptomatic vasospasm at the bedside. Here, we demonstrate that TD flowmetry allows the assessment of cerebral hemodynamic parameters, such as perfusion and vascular resistance, in SAH patients and reliably detects the development of vasospasm-associated hypoperfusion. Furthermore, we have determined the diagnostic cutoff values, predictive values, and likelihood ratios of TD flowmetry for identification of symptomatic vasospasm.

Clinical Material and Methods

Adult patients with thick SAH on a cranial CT study (n = 14 total, grade 3 to 5 according to the World Federation of Neurological Surgeons[4] classification and grade 3 according to the scale of Fisher and colleagues[5]) were included in the study All patients had ruptured anterior circulation saccular aneurysms detected by cerebral angiography and underwent uneventful surgical clipping of the aneurysm within 48 hours of rupture. Patients were enrolled prospectively. Routine monitoring of the patients included invasive measurement of mean arterial blood, central venous, intracranial, and cerebral perfusion pressures. Following aneurysm clipping, two TD microprobes (Hemedex Inc., Cambridge, MA, USA) were implanted into the vascular territories at highest risk of developing vasospasm-associated hypoperfusion. For example, in the case of an internal carotid or middle cerebral artery aneurysm the probes were implanted into the ipsilateral middle and anterior cerebral artery territories. The probes were inserted through a one-way bolt and placed subcortically at a depth of 20 to 25 mm below the level of the dura. Measurements of TD-rCBF were performed at a sampling rate of 1Hz (TDP200 Perfusion Monitoring System, Hemedex Inc., Cambridge, MA, USA). As a potential measure of vasospasm severity, cerebrovascular resistance (CVR = TD-rCBF/cerebral perfusion pressure) was calculated.[2]

Following surgical clipping and probe implantation, a CT scan and stable xenon-CT study were performed on all patients on the first day postoperatively. This allowed for detection of acute ischemic lesions and measurement of baseline stable xenon-rCBF values. During the subsequent monitoring period, data on mean arterial, intracranial, and cerebral perfusion pressures, TD-rCBF, CVR, and cerebral arterial blood flow velocities were collected at the bedside every 12 hours. According to the time course of vasospasm, a second stable xenon-CT study followed by cerebral angiography was performed between 7 and 9 days after SAH or earlier in cases of neurological deterioration or pathological transcranial Doppler (TCD) values. Symptomatic vasospasm was defined as a delayed ischemic neurological deficit or a stable Xenon rCBF < 32 mL/100 g/min on the second stable xenon-CT study in the presence of angiographic vasospasm.[6]

Results

For interpretation of the focal TD-rCBF data, it is important to note that the white matter CBF is expected to range between 18 and 25 mL/100 g/min, as compared with the mean global CBF, which is expected to be between 40 and 50 mL/100 g/min.[7] TD flowmetry nicely complemented the bedside monitoring of our SAH patient population. It became clear that, whereas both TCD and cerebral perfusion pressure represent only indirect and unreliable measures of cerebral perfusion, TD flowmetry enabled a direct assessment of cerebral hemodynamic parameters. Following an early period of posthemorrhage hypoperfusion (TD-rCBF = 18 ± 1 mL/100 g/min), TD-rCBF recovered to 23 ± 2 mL/100 g/min on days 2 and 3 after SAH, to finally decrease steadily from days 4 to 9 (TD-rCBF = 13 ± 1 mL/100 g/min), as expected for a population of patients with severe SAH.

CBF and CVR values were analyzed according to the clinical course of our study population which revealed distinct patterns for both parameters in patients with and without vasospasm. In patients without vasospasm, TD-rCBF remained > 20 mL/100 g/min for almost the entire monitoring period. On

the other hand, patients with vasospasm exhibited a gradual decrease in TD-rCBF to 10 ± 2 mL/100 g/min by the day of diagnosis of vasospasm. This gradual manifestation of symptomatic vasospasm also was reflected in the time course of change in CVR course. In patients with vasospasm, CVR gradually increased from a value of 7 ± 1 to 36 ± 11 (vs a CVR of < 7 in patients without vasospasm).

Next, we sought to determine a diagnostic cutoff value for TD-rCBF and CVR, which would be indicative of symptomatic vasospasm within the monitored vascular territory. Comparing the results of our stable xenon-rCBF measurements on the day of the diagnostic workup for vasospasm with the TD-rCBF results from the same day revealed a TD-rCBF cutoff value between 10 and 15 mL/100 g/min. Similarly, a comparison between CVR and the corresponding stable xenon-CT measurements revealed a CVR cutoff value between 10 and 12 for the diagnosis of symptomatic vasospasm. A further detailed statistical workup demonstrated that a TD-rCBF value of 10 mL/100 g/min and a CVR value of 10 would represent the best diagnostic thresholds because they minimize the sum of false-positive plus false-negative rates. It should be noted, however, that both cutoffs are associated with relevant false-negative rates, missing up to 13% of vascular territories with vasospasm-associated hypoperfusion. Therefore, to minimize the risk of false-negative misses, we prefer a TD-rCBF cutoff value of 15 mL/100 g/min, which suffers from a lower likelihood ratio for positive test results but provides the highest reliability in ruling out symptomatic vasospasm. This, however, will leave a diagnostic "gray zone" between a TD-rCBF of 10 mL/100 g/min and 15 mL/100 g/min. In this case, the additional calculation of the CVR becomes of special interest to identify those patients that are dependent on a high cerebral perfusion pressure to achieve this borderline perfusion. These patients may develop symptomatic vasospasm if cerebral perfusion pressure declines.

Finally, we sought to identify the reliability of TD flowmetry in identifying the patient with vasospasm. When a TD-rCBF cutoff value of 15 mL/100 g/min was applied to the two microprobes implanted in the vascular territories at risk, none of the patients with symptomatic vasospasm was missed by TD flowmetry (sensitivity 100%, specificity 75%). Interestingly, this result would have been only slightly worse in the case of only one probe implanted into the vascular territory at highest risk, where one patient with symptomatic vasospasm would have been missed (sensitivity 90%, specificity 75%). However, in comparison to the results obtained by using TCD criteria (sensitivity 80%, specificity 50%), both the unifocal and multifocal TD-rCBF monitoring provided a more favorable diagnostic reliability.

Conclusion

TD flowmetry represents a promising technique for continuous bedside monitoring and detection of symptomatic vasospasm in patients with SAH. This is of major interest for SAH patients that cannot be assessed clinically such as those that suffer from a severe hemorrhage, remain comatose, or have to remain sedated to control intracranial hypertension. Despite the minimal invasiveness of the technique, both the implantation of the microprobes as well as the monitoring procedure have proven to be safe in the high-grade SAH patient population. In addition to the quantitative assessment of rCBF, we have introduced a further parameter that can be assessed by TD flowmetry; namely CVR, which provides complementary bedside information on vasospasm severity by considering the cerebral perfusion pressure necessary to achieve a certain perfusion. The limiting character of the focal nature of rCBF measurements can be largely overcome by careful selection of the vascular territory to be monitored and following a standardized implantation protocol. In a next step, the results of this study have to be confirmed in a multicenter monitoring study of SAH patients using TD flowmetry.

REFERENCES

1. Thome C, Vajkoczy P, Horn P, et al. Continuous monitoring of regional cerebral blood flow during temporary arterial occlusion in aneurysm surgery. J Neurosurg 2001;95:402–411
2. Vajkoczy P, Horn P, Bauhuf C, et al. Effect of intra-arterial papaverine on regional cerebral blood flow in hemodynamically relevant cerebral vasospasm. Stroke 2001;32:498–505
3. Vajkoczy P, Roth H, Horn P, et al. Continuous monitoring of regional cerebral blood flow: experimental and clinical validation of a novel thermal diffusion microprobe. J Neurosurg 2000;93:265–274
4. Drake CG, Hunt WE, Sano K, et al. Report of World Federation of Neurological Surgeons Committee on a Universal Subarachnoid Hemorrhage Grading Scale. J Neurosurg 1988;68:985–986
5. Fisher CM, Kistler JP, Davis JM. Relation of cerebral vasospasm to subarachnoid hemorrhage visualized by computerized tomographic scanning. Neurosurgery 1980;6:1–9
6. Clyde BL, Resnick DK, Yonas H, et al. The relationship of blood velocity as measured by transcranial Doppler ultrasonography to cerebral blood flow as determined by stable xenon computed tomographic studies after aneurysmal subarachnoid hemorrhage. Neurosurgery 1996;38:896–904
7. Hoedt-Rasmussen K. Regional cerebral blood flow: the intra-arterial injection method. Acta Neurol Scand 1967;43(suppl 27):21–81

SECTION VII

Clinical—Medical Aspects

45

Sex and Clinical Cerebral Vasospasm in Yorkshire, UK

AUDREY C. QUINN, M.B.CH.B., F.F.A.R.C.S.I., KEN INWEREGBU, M.B.CH.B., B.SC, F.R.C.A., ANU SHARMA, M.B.B.S., D.A., F.F.A.R.C.S.I., LEONA BEECROFT, R.G.N., B.Sc, Jo GELDARD, R.G.N.DIP, SIMON THOMSON, M.B.B.S., F.R.C.S.(S.N.), ELIZABETH M. A. HENSOR, PH.D., ALAN TENNANT, B.A., PH.D., STUART ROSS, M.CH., F.R.C.S.(S.N.)

Abstract

Clinical cerebral vasospasm after subarachnoid hemorrhage (SAH) is a common complication and is associated with significant morbidity and mortality. We have audited our own figures regarding this condition. Over a 30-month period, 242 patients with a diagnosis of SAH were admitted to our neurosurgical unit in Leeds. There was a male:female ratio of 1:2.4. Seventy-seven patients were given the clinical diagnosis of vasospasm based on a fall in the Glasgow coma score of two or more points in the absence of sepsis, seizures, hydrocephalus, metabolic disorders, and rebleeding. Patients with vasospasm had an average age of 49 ± 10 years and a male:female ratio of 1:3.5. There were 20 deaths from vasospasm, and 18 of these patients were female, leading to a male:female ratio of 1:9. Approximately 50% of patients were smokers, 50% had a history of hypertension, and there were no differences in these frequencies between patients who died and those who survived. The data suggest a higher incidence of vasospasm in women. The reason for this remains unclear. A quarter of patients admitted with SAH developed clinical vasospasm in our patient group. There was a female predominance of incidence of SAH, vasospasm, and mortality in women. In this patient group there was no difference in outcome with respect to smoking, hypertensive history, or World Federation of Neurological Surgeons grade on the day of vasospasm.

The female predominance of patients with aneurysmal subarachnoid hemorrhage (SAH) has been known for some time.[1,2] The approximate male:female ratio has been reported as 1:2[3] with a similar mortality ratio.[4] Hormonal changes at the menopause have been proposed as an explanation for the gender difference.[5,6] A recent study indicated that hormone replacement therapy may have a protective role against SAH and suggested that low estrogen levels that occur during and after menopause may result in decreased collagen content within arterial vessels, and hence predispose to aneurysm formation.[7] However, the therapeutic

implications of these findings are uncertain in light of the increasing reluctance to prescribe hormone replacement therapy.[8,9]

The identification of risk factors or predictors for SAH and its common sequela, cerebral vasospasm, will undoubtedly be important in minimizing this crippling disease. Recent studies have presented several possible factors that may predict the development of vasospasm including thickness of subarachnoid clot on cranial computed tomography (CT) done acutely after SAH, early increase in transcranial Doppler flow velocities, Glasgow coma scale score < 15, presence of a carotid or anterior cerebral artery aneurysm, age < 50 years, good neurological grade (World Federation of Neurosurgeons grades 1 and 2) and hyperglycemia.[10–12] We report the findings of the initial 30 months of our prospective audit of SAH, which aims to identify possible risk factors for SAH and vasospasm as well as the effect of gender on outcome.

Patients and Methods

Study Population

West Yorkshire has a population of 2.5 million. Approximately 100 cases of aneurysmal SAH cases are seen annually at our instutition and our policy is to admit all grades. The treatment in our center is early surgical clipping. We have only one interventional radiologist and hence radiological intervention is a limited resource. At present the treatment of cerebral vasospasm is hemodynamic therapy (induced hypertension, hypervolemia, and hemodilution). The diagnosis of SAH is based on a head CT scan along with a lumbar puncture in doubtful cases. SAH on admission was classified on the Fisher scale.[13] A confirmatory cerebral angiogram is performed in the surgically treated cases. A diagnosis of vasospasm is made on the basis of clinical deterioration (drop of the Glasgow coma score of two or more points) plus increased transcranial Doppler (TCD) velocities. A head CT scan is performed after this decline to exclude other possible causes of deterioration such as rebleeding, infection, and hydrocephalus. In our prospective audit we recorded all deaths where vasospasm was implicated as a cause of death or was deemed to be a major contributing factor.

Data Collection

Baseline demographic data were collected on the day of admission of all SAH patients over a 30-month period. A range of clinical and biochemical data were collected on a daily basis by the trained senior nurse. A total of 908 variables were collected per patient over the first 14 days after admission. A subset of this data in two files is relevant to this audit (Table 45–1). There is an admission database of patients with SAH and a vasospasm database of the patients who developed this complication.

Statistical Analysis

All data were collected and analyzed using statistical computer software (Statistical Package for the Social Sciences [SPSS], Chicago, IL, USA). The variables are recorded in Table 45–2. One linear regression model was developed to predict the occurrence of vasospasm and another to predict death from vasospasm. This was achieved in each case by first entering all relevant variables into a stepwise linear regression model and identifying those that were significant (or borderline significant, $p < .1$) predictors. These selected variables were then entered into a simultaneous linear regression model.

Results

Between November 1999 and May 2003, 242 patients were entered prospectively. These cases formed the admissions database. Seventy-seven patients had vasospasm (vasospasm database). Overall mortality at 14 days was 20%, with 9% of this due to vasospasm. The demographic data are presented in Table 45–3. There was a female predominance in the incidence of vasospasm and in subsequent death in our population.

Admission Database

Variables generated in our model that predicted vasospasm were female sex, loss of consciousness at

TABLE 45–1 List of Parameters Recorded in the Admission and Vasospasm Databases

SPSS File	Variables
Admission Database	Presenting complaint, past medical history, common grading scores, neuroradiology findings, hematology and biochemistry results, baseline observations, and hemodynamics
Vasospasm Database	Details of patients developing clinical cerebral vasospasm including day of onset, management, and outcome, including death where vasospasm was judged to be a major contributing factor

TABLE 45–2 Variables Entered into Initial Multivariate Analysis Models

	Admission Database	Vasospasm Database
Demographics	Sex, age, day of admission after SAH, Glasgow coma score at base hospital and on admission to Leeds, World Federation of Neurological Surgeons grade	World Federation of Neurological Surgeons Grade and Glasgow coma score on day of and day after onset of vasospasm, gender, age, day of onset after SAH
History	Loss of consciousness, meningism, cranial nerve palsy, seizures, hemiparesis, headache, visual impairment, previous SAH, smoking status, hypertension, on treatment for hypertension, respiratory, cardiovascular, renal and hepatic disease, diabetes, migraine	Day of and day after spasm: history of hypertension, smoker
Radiology	Computed tomographic diagnosis of hydrocephalus, Fisher grade, multiple aneurysms, anterior circulation aneurysm, anterior communicating artery aneurysm	Angiographic confirmation of vasospasm, presence of clip on aneurysm
Treatment factors	External ventricular drain, dose, and administration of nimodipine	Inotrope therapy, 24-hour fluid balance, dose and mode of nimodipine administration
Physical examination	Admission heart rate, mean arterial blood pressure, central venous pressure, temperature	Heart rate, central venous pressure, mean arterial blood pressure, temperature
Laboratory values	Hemoglobin, white blood cell count, platelet count, international normalized ratio, serum sodium potassium, urea, creatinine, glucose, lactate, fractional inspired oxygen, pO_2,pCO_2,pH	Hemoglobin, platelet count, serum sodium, glucose, urea, creatinine, pO_2, pCO_2, pH, lactate, international normalized ratio, lactate

SAH, subarachnoid hemorrhage.

onset of SAH, patient admitted on day of SAH, cranial nerve palsy, headache or hypothermia on admission, presence of hydrocephalus on CT, necessity for external ventricular drainage, presence of multiple aneurysms, and anterior communicating aneurysm. Using these factors, we were able to predict whether a patient subsequently developed vasospasm in 82% of SAH cases, correctly identifying 64% of vasospasm cases.

Vasospasm Database

Variables produced by the model that predicted death were dose of intravenous nimodipine, and low serum sodium, pO_2, and pCO_2. Those patients receiving intravenous nimodipine at 5 or 10 mL/hour were predicted to die more frequently than those on oral nimodipine. This model predicted outcome (survival or death) in 80% of cases correctly while being able to predict 40% of all deaths in the vasospasm group.

Discussion

The preliminary results of this audit demonstrate an overall mortality at 28 days of 20%. There may be a higher proportion of deaths from vasospasm than reported in some other series. This audit has raised

TABLE 45–3 Demographic Data

Group	Male	Female	Gender Ratio (M:F)
Subarachnoid hemorrhage (n = 242)	72 (mean age 51 years)	170 (mean age 51 years)	**1:2.4**
Vasospasm (n = 77)	17 (mean age 49 years)	60 (mean age 49 years)	1:3.5
Death from vasospasm (n = 20)	2	18	**1:9**
World Federation of Neurological Surgeons grades 1 and 2	48	109	1:2.1
World Federation of Neurological Surgeons grades 3 and 4	15	38	1:2.5
Anterior circulation aneurysm	46	111	1:2.4
Posterior circulation aneurysm	8	20	1:2.5

many questions requiring further analysis. Our main methodological concerns are whether we are correctly defining vasospasm and whether we are justified in implicating vasospasm as the cause of death in patients with other life-threatening pathologies. We have noted a gender difference in the incidence and also in the rate of death from vasospasm, although an obvious explanation for this difference is not evident from the data. Sex was a significant variable in the predictive model only in the admission database despite the wide gender difference in the vasospasm file. This is presumably because the numbers of male deaths are too small to allow comparisons with the female deaths.

We feel uncomfortable with the definition of clinical vasospasm that is one of exclusion, and have tried to minimize the subjectivity by ensuring that the senior research nurse involved has ready access to senior medical staff for confirmation of the diagnosis. In addition, recording death from vasospasm can be unclear owing to the coexistence of other pathologies. The clinical notes of the patient who died were retrieved and examined at the time of data analysis to assure the investigators that vasospasm played a major part in the death. The presence of anterior communicating aneurysm was a significant predictor of vasospasm in our SAH population. Qureshi et al classified aneurysms in their study into two groups: (1) anterior cerebral or internal carotid arteries and (2) posterior circulation or middle cerebral arteries.[11] They found an aneurysm in the first group to be a predictor of vasospasm but did not give any specific information about the anterior communicating arteries in their patients. One assumes them to be included in this group. Charpentier and coworkers did provide a breakdown of their aneurysm location but did not find a relationship between location of aneurysm and vasospasm.[10] It is necessary that anatomical location be closely and accurately defined to enable comparison between centers because it seems to be important in predicting patients likely to develop vasospasm.

We were interested to observe that factors from the patients' medical history and presenting symptoms may be predictive of vasospasm, such as loss of consciousness at time of SAH, cranial nerve palsies, headache, and hypothermia on admission. Some of these features may be a reflection of the severity of the original insult. The primary injury is increasingly being recognized as the important determinant of outcome because secondary insults are being minimized by careful medical management after admission. In our institution SAH patients with good grades are often nursed in a general neurosurgical ward where monitoring is minimal and surveillance and detection of vasospasm may not be as rapid as in intensively nursed patients. This fact emphasizes the importance of methods to predict those patients at risk so they may be managed in the appropriate hospital setting. The presence of hydrocephalus on the admission CT and the consequential need for an external ventricular drain were predictive of vasospasm in our population. This is a controversial area in our institution because there is a concern that rebleeding results from the release of a "tamponade" effect over a ruptured aneurysm when an external ventricular drain is inserted. In contrast, the insertion of a drain is also thought to be helpful in low grade patients. We plan to look more closely at our diagnosis and management of acute hydrocephalus in our patients.

Our audit has also highlighted that death from vasospasm may be associated with the use of intravenous nimodipine and low serum sodium, pO_2, and pCO_2. These features are common to our sicker patients and they require closer examination. We have been conscious of the preliminary nature of this study. This has been the first analysis of the data, and we are striving to refine the data and develop the best statistical analysis from our database. We are currently setting up a follow-up system to carefully assess the longer term outcome and morbidity of these patients, which we hope will clarify the true outcome of this lethal disease, and we plan to continue this audit for the foreseeable future.

Conclusion

Of 242 prospective aneurysmal SAH admissions, we documented that 77 had cerebral vasospasm and that 20 died as a consequence. Our data indicate that previously reported risk predictors were not of value in predicting vasospasm in our patients.[8,9] We believe this is a reflection of the heterogeneity of the study population. Female sex is a significant risk factor determining the onset of vasospasm in our population. We do not yet have enough patients to analyze the effect of sex on eventual death from vasospasm. We plan to examine more closely the predictive variables from this interim analysis of our audit and their relevance to the diagnosis and death from vasospasm.

REFERENCES

1. Bonita R, Thomson S. Subarachnoid hemorrhage: epidemiology diagnosis, management and outcome. Stroke 1985;16:591–594
2. Ostergaard JR, Hog E. Incidence of multiple intracranial aneurysms: influence of arterial hypertension and gender. J Neurosurg 1985;63:49–55
3. Andrews RJ, Spiegel PR. Intracranial aneurysms: age, sex, blood pressure and multiplicity in an unselected series of patients. J Neurosurg 1979;51:27–32
4. ACROSS Group. Epidemiology of aneurysmal subarachnoid hemorrhage in Australia and New Zealand: incidence and case

fatality from the Australian Cooperative Research on subarachnoid haemorrhage Study (ACROSS). Stroke 2000;31:1843–1850
5. Longstreth WT, Nelson L, Koepsell T, Belle G. Subarachnoid hemorrhage and hormonal factors in women. Ann Intern Med 1994;121:168–173
6. Mhurchu CN, Anderson C, Jamrozik K, Hankey G, Dunbabin D. Australasian Cooperative Research on Subarachnoid Hemorrhage Study (ACROSS) Group: hormonal factors and risk of aneurysmal subarachnoid hemorrhage: an international population-based, case-control study. Stroke 2001;32:606–612
7. Kongable G, Lanzino G, Germanson TP. Gender related differences in aneurysmal subarachnoid hemorrhage. J Neurosurg 1996;84:43–48
8. Olsson H, Ingvar C, Bladstrom A. Hormonal replacement therapy containing progestins and given continuously increases breast carcinoma. Cancer 2003;97:1387–1392
9. Stephenson J. FDA orders estrogen safety warnings: agency offers guidance for HRT use. JAMA 2003;289:537–538
10. Charpentier C, Audibert G, Guillemin F, et al. Multivariate analysis of predictors of cerebral vasospasm occurrence after aneurysmal subarachnoid hemorrhage. Stroke 1999;30: 1402–1408
11. Qureshi AL, Sung GY, Razumovsky AY, Lane K, Straw RN, Ulatowski JA. Early identification of patients at risk for symptomatic vasospasm after aneurysmal subarachnoid hemorrhage. Crit Care Med 2000;28:984–990
12. Drake CG, Hunt WE, Sano K, et al. Report of World Federation of Neurological Surgeons Committee on a Universal Subarachnoid Hemorrhage Grading Scale. J Neurosurg 1988;68:985–986
13. Fisher CM, Kistler JP, Davis JM. Relation of cerebral vasospasm to subarachnoid hemorrhage visualized by computerized tomographic scanning. Neurosurgery 1980;6:1–9

46

Factors Influencing Vasospasm After Aneurysmal Subarachnoid Hemorrhage: A Prospective Observational Study in the North of England

RAMANATHAN VENKATACHALAM, F.R.C.S., LINDA P. HUNT, PH.D., ALAKANDY M. LIKHITH, F.R.C.S., N. T. GURUSINGHE, G. A. ROBERTS, CHARLES H. G. DAVIS, F.R.C.S., RICHARD H. LYE, PH.D., F.R.C.S.

Abstract

Cerebral arterial vasospasm or delayed ischemic neurological deficit continues to be one of the major causes of morbidity and mortality following aneurysmal subarachnoid hemorrhage (SAH). Even though advances have been made in the effective management of aneurysms, there has been little advance made in understanding the factors involved in the causation and maintenance of cerebral vasospasm. This study was designed to look at a variety of clinical and laboratory variables in an unselected series of consecutive patients with aneurysmal SAH in the north of England. The objectives were to search for factors that predicted nonhemorrhagic deterioration and to examine overall outcome in an unselected population with aneurysmal SAH. A prospective observational study was undertaken of all patients referred with proven SAH to four neurosurgical units in the north of England between May 1, 1989, and October 31, 1992. The only modality of aneurysm treatment was surgery. Demographic, clinical, and radiological data collected through pro forma and laboratory data from human leukocyte antigen (HLA) typing were entered into a computer and analyzed using statistical software. There were 462 patients enrolled in the study, of which 92% had anterior circulation aneurysms and 97% were given nimodipine. Surgery was performed on 91% of patients at a mean of 9 days after SAH (range: 4–14 days). The overall management mortality was 10%, of which 3% was due to rebleeding. The overall rehemorrhage rate was 5%. Seventy-six percent of patients were classified as good recovery or moderate disability on the Glasgow outcome scale at discharge. The incidence of vasospasm was 34%, and vasospasm was transient in 12%. History of hypertension, increasing age, premonitory headache, higher neurological grade, and grade of SAH on computed tomography were associated with a statistically significant increased risk of developing vasospasm. The presence of HLA CW8 and B14 appeared to reduce the risk of developing vasospasm, whereas the presence of HLA CW4 increased it. A statistical model for prediction of vasospasm was developed from the risk factors.

Survival analysis showed that those with a hazard ratio of > 3.2 had a > 35% chance of developing vasospasm by 30 days, and those with a ratio of < 1.5 had a 19% chance. There was a baseline hazard for vasospasm even in the absence of risk factors. Vasospasm continues to be a major factor affecting outcome after SAH. The presence of premonitory headaches was associated with a higher risk of developing vasospasm. The use of a statistical model could be useful for vasospasm prediction in the individual patient.

Over the past 10 years advances in the management of cerebral aneurysms have revolutionized neurosurgery. This includes advances in microsurgery, neuroanesthesia, and endovascular techniques. However, we still know very little about cerebral arterial vasospasm despite a vast amount of literature on the subject.[1] Delayed ischemic neurological deficits or symptomatic vasospasm are known to occur in 17 to 40% of patients with aneurysmal subarachnoid hemorrhage (SAH).[2] Vasospasm is the leading cause of mortality and morbidity in these patients.[2] It is currently unclear whether endovascular treatment has altered the occurrence of vasospasm.

This study examined a variety of clinical and laboratory factors in an unselected population with aneurysmal SAH to identify factors that might predict the occurrence of vasospasm.

Materials and Methods

The study recruited 462 patients with aneurysmal SAH who presented to the four neurosurgical departments in Manchester and Preston in the north of England between May 1, 1989, and October 31, 1992. The criteria for inclusion were SAH confirmed by cranial computed tomography (CT) or lumbar puncture and aneurysm confirmed at angiography or autopsy or a hematoma on the CT that was secondary to an aneurysm. The study was noninterventional in that the clinical management of the patients in the study was left strictly to the discretion of the individual units. Participating neurosurgeons completed detailed proformas for each patient at the time of admission to the unit and at discharge. Nursing staff completed a "ward diary" for the duration of the time spent by each patient on the unit.

Results

Of the 462 patients, 97% were Caucasian. The median age was 49 years (range: 17–73 years), with 63% being female. Sixty-five percent had smoked within the last 6 months and 18% had a history of hypertension requiring treatment. Symptoms suggestive of SAH ("thunderclap headache") within a month of the ictus were recorded in 7% of cases (33%), and 26% had a premonitory headache a week or so before the ictus. Ninety-seven percent of patients received nimodipine. In 10% of patients the serum sodium was below the reference limit (< 132 mmol/L) at admission. CT scans were performed on all but 14 patients (3%). Surgery was performed on 91% of the patients at a median time of 9 days from the ictus (interquartile range 4–14 days). Seventy-six percent (353 patients) were classified as good outcomes (good recovery or moderate disability on the Glasgow outcome scale[3] at discharge, Glasgow outcome scale score of 4 or 5), whereas 24% (109 patients) had a "poor" outcome (severe disability, vegetative state, or dead, Glasgow outcome score of 1 to 3). The World Federation of Neurological Surgeons (WFNS) grade[4] was 1 or 2 in 83% of patients on admission, and 83% of these patients had a good outcome (Glasgow outcome scale score of 4 or 5). Of the 80 patients admitted with poor WFNS grades (3–5), 48% had a good outcome, and of the 45 patients in WFNS grades 4 and 5, 38% had a good outcome. Death occurred in 44 patients (10%), of which 14 were from rehemorrhage (3%), 20 from nonhemorrhagic deterioration (4%), and 10 from other causes (2%). There were seven patients who rebled but survived. The overall rebleed rate was 5%. The overall incidence of delayed ischemic neurological deficit was 34% (157 patients), with an additional 12% (54 patients) having transient episodes of delayed ischemia. Three-month follow-up was obtained in 69% of cases, and 88% of these had a good outcome. Seven additional deaths were reported at 3 months, of which three were due to recurrent hemorrhage related to the presenting aneurysm and one to hemorrhage from a previously unruptured aneurysm.

Multivariate analysis using logistic regression showed that overall outcome was significantly related to age ($p = .007$) and WFNS score at admission ($p < .001$). Multivariate analysis showed that older age ($p = .004$), presence of premonitory headache ($P = .016$), lower serum sodium at admission ($p = .028$), higher WFNS grade ($p = .017$), and higher grade of SAH on CT scan as graded on the Fisher scale[5] ($p = .002$) were

significantly related to the occurrence of delayed ischemic neurological deficit. The presence of human leukocyte antigen (HLA) B14 and Cw8 (risk ratios 0.14 and 0.15, respectively) were associated with a lower risk of delayed ischemia, and the presence of HLA Cw4 (risk ratio 2.0) was associated with a higher risk of delayed ischemia. The coefficients for the preceding factors (except HLA) were used to obtain a score to estimate a patient's relative risk (hazard rate ratio) of developing delayed ischemia within 30 days. Risks were relative to those of a baseline patient of age 48 years with no premonitory headache, serum sodium 137 mmol/L, WFNS grade 1, and no SAH seen on CT scan (Fisher grade 1). A plot of the baseline hazard rate for the preceding factors shows a peak approximately 8 days after SAH (Fig. 46–1). After adjustment for timing of operation using, in addition to the above baseline patient characteristics, not undergoing surgery, the peak remained.

A predictive scoring system was derived using the coefficients of the factors obtained on multivariate analysis for delayed ischemic neurological deficit. This was calculated as follows: Score = exp [(observed age - 48) X coefficient of age] + [coefficient of premonitory headache] + [(observed sodium - 137) X coefficient of serum sodium] + [coefficient of WFNS grade] + [coefficient of CT grade]. Scores were estimates of risk relative to the baseline category (as already defined) that had a risk set at 1. The estimated percent that did not develop delayed ischemia at 30 days in the baseline category was 87%. The cumulative probability of delayed ischemic deficit in this group, therefore, was 13%. Estimates of the cumulative probability for other patients could be calculated from their scores using the formula: $1 - 0.874^{Score}$.

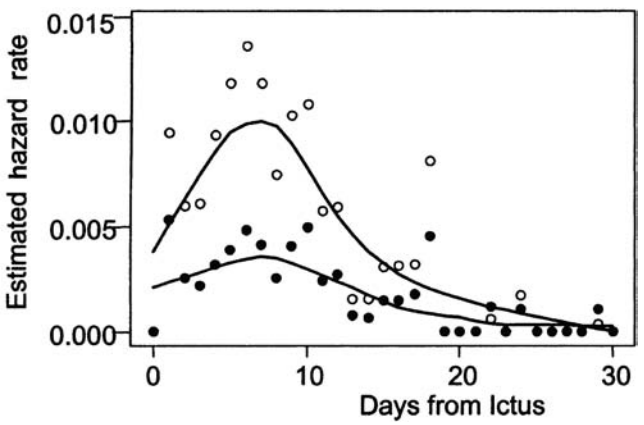


FIGURE 46–1 Smoothed estimates of the baseline hazard rate for development of delayed ischemic neurological deficit for all factors significantly associated with delayed ischemia in multivariate logistic regression (upper curve) and after adjustment for timing of surgery (lower curve).

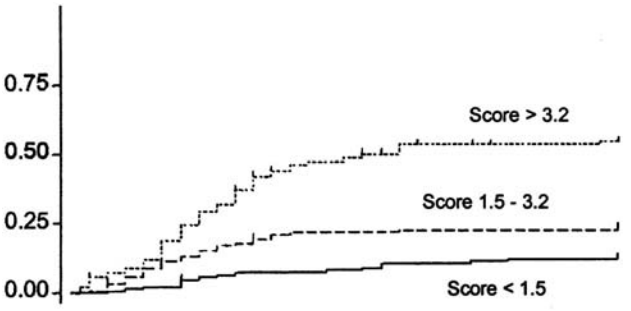


FIGURE 46–2 Kaplan-Meier estimates of cumulative risk of delayed ischemic neurological deficit for patients divided into three groups on the basis of their expected risk derived from multivariate regression analysis.

As an example a patient age 47 years, with no headache, WFNS grade 3, CT grade 1, and serum sodium 131 would have a score of 3.0 and therefore would be expected to have a 33% chance of developing vasospasm in the first 30 days. The whole cohort of patients was ranked in order of ascending scores and divided into tertiles. The tertiles were calculated to be 1.5 and 3.2, corresponding to estimated chances of developing delayed ischemic deficits of 19% and 35%, respectively. Patients with scores < 1.5 or > 3.2 would be expected to have < 19% risk or > 35% risk, respectively. Kaplan-Meier estimates of the cumulative probability of delayed ischemic neurologic deficit for these subgroups are shown in Figure 46–2.

Conclusion

Premonitory headaches were associated with a higher risk of cerebral vasospasm. The presence of HLA B14 and Cw8 was associated with a lower occurrence and HLA Cw4 with a higher occurrence of clinical vasospasm. A scoring system has been developed to predict the occurrence of clinical vasospasm, which may be useful to predict vasospasm in an individual patient because the population in this study is fairly representative of the general population of aneurysmal SAH.

REFERENCES

1. Macdonald RL, Weir BK. Cerebral Vasospasm. San Diego: Academic; 2001:1–518
2. Dorsch NWC, King MT. A review of cerebral vasospasm in aneurysmal subarachnoid hemorrhage, I: Incidence and effects. J Clin Neurosci 1994;1:19–26
3. Jennett B, Bond M. Assessment of outcome after severe brain damage: a practical scale. Lancet 1975;1:480–484
4. Drake CG, Hunt WE, Sano K, et al. Report of World Federation of Neurological Surgeons Committee on a Universal Subarachnoid Hemorrhage Grading Scale. J Neurosurg 1988;68:985–986
5. Fisher CM, Kistler JP, Davis JM. Relation of cerebral vasospasm to subarachnoid hemorrhage visualized by computerized tomographic scanning. Neurosurgery 1980;6:1–9

47

Grading Scale for Subarachnoid Hemorrhage Based on a Modification of the World Federation of Neurological Surgeons Scale

DAVID S. ROSEN, M.D. AND R. LOCH MACDONALD, M.D., PH.D.

Abstract

Physicians treating patients with subarachnoid hemorrhage (SAH) use data from the patient's history, physical examination, and radiological studies to make clinical management decisions. However, none of the commonly used SAH grading systems includes historical or radiological data. Our work examines the prognostic significance of clinical parameters included in a patient's history, presentation, and radiological studies and derives an SAH grading scale based on the most powerful prognostic parameters. We analyzed a database containing 3567 patients with SAH who were entered into four clinical trials of tirilazad. These prospective, randomized, double-blind, placebo-controlled clinical trials were conducted in neurosurgical centers between 1991 and 1997. Outcomes were assessed at 3 months post-SAH using the Glasgow outcome scale. Twenty clinical parameters were investigated. A new SAH grading system was statistically derived and validated. The new SAH grading system was compared with the World Federation of Neurological Surgeons (WFNS) scale using a receiver operating characteristics curve. Analysis of the WFNS scale and 19 additional clinical parameters for prognostic strength revealed that, in addition to the WFNS scale, seven parameters are significant and 12 are not. The seven clinical parameters include age, history of hypertension, systolic blood pressure, ruptured aneurysm location, ruptured aneurysm size, blood clot thickness, and presence of angiographic vasospasm on admission. We statistically derived and validated a modification to the WFNS scale based on these additional parameters. A comparison of our modified scale to the standard WFNS scale revealed that the new scale had stronger prognostic power. Our modification to the WFNS scale gives the clinician additional prognostic power in evaluating patients with SAH.

The outcome of patients with subarachnoid hemorrhage (SAH) from ruptured intracranial aneurysms will be influenced by factors related to the patient, the pathology, and the treatments rendered. These factors may be clinical, biochemical, or radiological and in terms of treatment, related to either or both medical and surgical maneuvers. Multivariate analyses of large populations of patients with aneurysmal SAH are few in number but demonstrate that the factors that exert the largest effects on outcome are patient

age, neurological grade, amount of SAH on admission computed tomographic (CT) scan, presence of intracerebral or intraventricular hemorrhage, aneurysm site and size, history of hypertension, and vasospasm.[1–3]

This work used the 3567 prospectively collected patients entered into studies of tirilazad to test the prognostic ability of the WFNS scale as well as 19 additional clinical parameters. A grading system was derived using clinical and radiological parameters. Finally, the prognostic ability of the new scale was compared with the WFNS scale.

Patients and Methods

A database containing 3567 patients with SAH who were entered into four clinical trials of tirilazad was analyzed.[4–7] These prospective, randomized, double-blind, placebo-controlled clinical trials were conducted in neurosurgical centers in Europe, Australia, New Zealand, South Africa, and North America between 1991 and 1997. Inclusion criteria included the presence of an angiographically documented saccular aneurysm causing SAH. SAH was confirmed by CT or lumbar puncture. Patients were at least 18 years of age and had to begin treatment within 48 hours of SAH. Exclusion criteria included nonsaccular aneurysms (fusiform, traumatic, or mycotic); severe complicating medical, neurological, or psychiatric illness; serious cardiovascular complications; pregnancy or lactation; use of steroids or calcium channel blockers; and placement of Guglielmi detachable coils in the ruptured aneurysm.

Patients were randomized to receive vehicle or various doses of tirilazad until day 10 after SAH. The mean time to aneurysm repair surgery was 55 hours after SAH. The treating physician was permitted to use nimodipine, hemodynamic therapy, and mechanical or pharmacological angioplasty but other experimental therapies such as steroids and other calcium channel antagonists were not allowed. Outcomes were assessed 3 months post-SAH using the five-point Glasgow outcome scale (GOS).

The entire database was randomly sorted into two groups for split sample validation. One group (training sample) was used to derive the grading scale, and the new scale was tested on the second group (validity sample). The prognostic significance of each of 19 variables was assessed by univariate logistic regression using a dichotomous outcome where favorable outcome was good recovery or moderate disability on the GOS and unfavorable outcome was severe disability, vegetative, or dead. Three parameters were not significantly associated with outcome and were excluded from the subsequent analysis. Multivariate analysis using backward selection was conducted on the remaining 16 parameters. Eight variables were predictive of outcome and theoretically could be used in a grading system. Changes in coefficients of ~0.5 were selected as one unit for the grading scale. This step allowed continuous variables such as age and aneurysm size to be collapsed into categories. A grading scale then was created based on collapsed variables.

The new grading system and the WFNS scale were then tested on the validity sample. To compare the various grading systems, Somers's D rank correlation and receiver operating characteristics (ROC) curves were calculated.

Results

Nineteen clinical and radiographic parameters were examined. Gender, race, level of consciousness, hydrocephalus, admission temperature, diastolic blood pressure, time to admission, intraventicular blood, diabetes history, history of hepatic disease, and presence of an intracerebral hematoma were not significant. A new SAH grading scale was created (Table 47–1). Only patients in grades 1 through 12 were present in the validity sample (Table 47–2). The likelihood ratio test shows a linear increase in risk of unfavorable outcome with

TABLE 47–1 Grading Scale Using Eight Clinical and Radiological Factors

Points	WFNS Grade	Age	History of Hypertension	Admission Systolic Blood Pressure	Aneurysm Size	Aneurysm Location	Clot Thickness	Admission Vasospasm
0	—	<50	**no**	**< 190 mmHg**	≤12 mm	anterior	**none or thin**	**no**
1	1	50–69	yes	**= 190 mmHg**	13–24 mm	posterior	**thick**	yes
2	2	70–79			≥ 25 mm			
3	3	≥ 80						
4	4							
5	5							

WFNS, World Federation of Neurological Surgeons.

TABLE 47–2 Prognostic Accuracy of World Federation of Neurological Surgeons and New Grading Scales Based on 1635 SAH Patients in Validity Sample

Grade	*N*	Favorable Outcome	Unfavorable Outcome	Likelihood Ratio
WFNS grade				
1	582 (33%)	506	76 (13%)	0.36
2	483 (27%)	386	97 (20%)	0.61
3	205 (12%)	118	87 (42%)	1.78
4	172 (10%)	85	87 (51%)	2.47
5	193 (11%)	61	132 (68%)	5.22
New grading scale				
1	96 (5%)	93	3 (3%)	0.08
2	192 (11%)	179	13 (7%)	0.18
3	285 (16%)	251	34(12%)	0.33
4	264 (15%)	212	52 (20%)	0.59
5	261 (15%)	182	79 (30%)	1.05
6	218 (12%)	127	91 (42%)	1.73
7	155 (9%)	70	85 (55%)	2.93
8	108 (6%)	28	80 (74%)	6.90
9	43 (2%)	9	34 (79%)	9.12
10	9 (0.5%)	4	5 (56%)	3.02
11	3 (0.2%)	1	2 (67%)	4.83
12	1 (0.06%)	0	1 (100%)	

each additional grade. The distribution of patients among the grades in the validity sample differs depending on the scale used with 64% of the patients in the validity sample being WFNS grades 1 or 2, whereas only 16% were grades 1 and 2 on the new scale.

The WFNS and the new grading scale were compared using ROC curves (Fig. 47–1). The ROC curve plots the probability of false-positives (1-specificity) on the x-axis and the probability of true positives (sensitivity) on the y-axis. The ROC for the new grading scale had a significantly greater area under it compared with the ROC for the WFNS scale (0.78 vs 0.74, $p < 0.05$). Therefore, at any given probability of a false-positive (x-axis), the new grading system will have a higher probability of identifying true-positives or unfavorable outcome (*y*-axis).

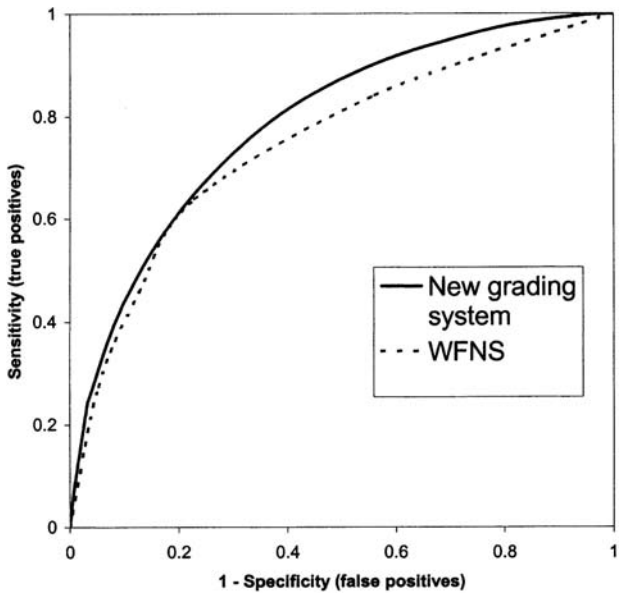


FIGURE 47–1 Receiver operating characteristic curve comparing the World Federation of Neurological Surgeons (WFNS) and new grading scales

Discussion

The patients entered into the studies of tirilazad were used to study methods of grading patients with aneurysmal SAH. The new result reported is that increased prognostic accuracy of grading can be achieved by incorporating additional clinical and radiographic factors into the grading system. Outcome prediction remains inexact, however, and it could be argued that only in a few situations can the outlook be judged in advance to be so bleak that treatment could reasonably be withheld.

Many authors use the Hunt and Hess scale to report clinical results. Although this scale was not specifically studied here, its advantages and limitations need to be considered in any discussion of grading scales. The subjective terms used to assess consciousness contribute to high interobserver variability. Second, discrimination in outcome between the grades may be poor, particularly between grades 1 and 2.

Factors used to grade patients on the Hunt and Hess scale, such as meningeal signs and headache, have no prognostic significance. The scale places patients in a higher grade if they have various medical illnesses but this is not consistently done in clinical practice, despite evidence from this and other studies that such factors are prognostically important. Finally, the Hunt and Hess scale was not statistically derived and was validated with mortality as the only end point.

The WFNS scale was proposed to improve SAH grading. Advantages include the use of the Glasgow coma score, which has less interobserver variability, at least when assessed by experienced individuals. The WFNS scale, however, was not statistically derived and instead was the product of expert opinion. In addition, differences in outcome between some grades may be minimal or absent although in this analysis, differences between all grades were present.

The ROC curve (see Fig. 47–1) shows that the prognostic accuracy of the new grading scale was higher than the WFNS scale. There were significant differences in outcome between each adjacent grade up to grade 8, after which the power substantially decreased. Most of the criteria are relatively objective and easy to assess. Some limitations should be acknowledged. Adding factors increases the complexity of the scale, and the likelihood of missing data increases as well. A CT scan and angiogram are required to grade patients using the new scale. It may be desirable to estimate prognosis prior to angiography in cases where a poor outcome seems very likely.

A newly recognized factor for unfavorable outcome after SAH is the presence of angiographic vasospasm at the time of admission. What this represents is unclear. Measurement of angiograms of patients with aneurysmal SAH shows that there is a graded reduction in arterial diameter after SAH that peaks 7 or 8 days after SAH and then resolves. Therefore, vasospasm on admission suggests that there was a prior unrecognized SAH or that there was arterial narrowing for some other reason, such as preexisting atherosclerosis, increased intracranial pressure, or stretching of arteries around a hematoma. The negative prognostic value of the narrowing may reflect these underlying conditions or adverse effects of the arterial narrowing itself.

REFERENCES

1. Kassell NF, Torner JC, Haley EC Jr, Jane JA, Adams HP, Kongable GL. The International Cooperative Study on the Timing of Aneurysm Surgery, I: Overall management results. J Neurosurg 1990;73:18–36

2. Kassell NF, Torner JC, Jane JA, Haley EC Jr, Adams HP. The International Cooperative Study on the Timing of Aneurysm Surgery, II: Surgical results. J Neurosurg 1990;73:37–47

3. Longstreth WT Jr, Nelson LM, Koepsell TD, van Belle G. Clinical course of spontaneous subarachnoid hemorrhage: a population-based study in King County, Washington. Neurology 1993;43: 712–718

4. Haley EC Jr, Kassell NF, Apperson-Hansen C, Maile MH, Alves WM. A randomized, double-blind, vehicle-controlled trial of tirilazad mesylate in patients with aneurysmal subarachnoid hemorrhage: a cooperative study in North America. J Neurosurg 1997;86:467–474

5. Kassell NF, Haley ECJ, Apperson-Hansen C, Alves WM. Randomized, double-blind, vehicle-controlled trial of tirilazad mesylate in patients with aneurysmal subarachnoid hemorrhage: a cooperative study in Europe, Australia, and New Zealand. J Neurosurg 1996;84:221–228

6. Lanzino G, Kassell NF. Double-blind, randomized, vehicle-controlled study of high-dose tirilazad mesylate in women with aneurysmal subarachnoid hemorrhage, II: A cooperative study in North America. J Neurosurg 1999;90:1018–1102

7. Lanzino G, Kassell NF, Dorsch NW, et al. Double-blind, randomized, vehicle-controlled study of high-dose tirilazad mesylate in women with aneurysmal subarachnoid hemorrhage, I: A cooperative study in Europe, Australia, New Zealand, and South Africa. J Neurosurg 1999;90:1011–1017

48

Effect of Inotrope-Induced Blood Pressure Changes on Cerebral Perfusion in Patients with Subarachnoid Hemorrhage: A Positron Emission Tomography Study

PAWAN S. MINHAS, M.D., PIOTR SMIELEWSKI, PH.D., DAVID K. MENON, M.B.B.S., M.D., PH.D., F.R.C.P., F.R.C.A., F.MED.SCI, MAREK CZOSNYKA, PH.D., PETER J. KIRKPATRICK, M.B., CH.B., F.R.C.S. (S.N.), JOHN D. PICKARD, M.D.

Abstract

Although the treatment of patients with delayed neurological deficits after aneurysmal subarachnoid hemorrhage (SAH) with hypertensive, hypervolemic, and hemodilutional (triple-H or hemodynamic) therapy is of proven value in reversing some ischemic deficits, the relative risk:benefit ratio for the use of various vasoactive inotropes is poorly defined. This study examined the effect of norepinephrine and dopamine, which were used to augment blood pressure in such patients, on cerebral blood flow measured with positron emission tomography (PET). The study included 20 patients with SAH. PET cerebral blood flow scans were performed at a mean initial arterial blood pressure of 88 mmHg and then at a mean arterial pressure of 105 mmHg that was achieved with dopamine (nine patients) or norepinephrine (11 patients). There were no significant changes in global cerebral blood flow with either dopamine or norepinephrine. Frequency histogram analysis revealed no significant regional changes in PET cerebral blood flow. In conclusion, in SAH patients receiving aggressive fluid management and vasoactive inotropic support to maintain mean arterial blood pressure at ~90 mmHg, a further increase in blood pressure to ~110 mmHg with the use of dopamine or norepinephrine does not produce significant change in global or regional cerebral blood flow. A larger study is required to identify whether a subgroup of patients may experience more profound effects on cerebral perfusion at the higher mean arterial pressures.

Although the etiology of delayed neurological deficits following aneurysmal subarachnoid hemorrhage (SAH) remains poorly understood, the incidence of such deficits and associated poor outcome has been reduced by the routine prophylactic use of nimodipine.[1] Cerebrovascular autoregulation is very frequently disturbed in patients who develop such deficits.[2] In keeping with that concept, hypertensive, hypervolemic, and hemodilutional (triple-H or hemodynamic) therapy using aggressive fluid resuscitation and vasoactive inotropes to augment blood pressure has been of benefit in reversing some ischemic deficits.[3–5] Optimizing such hemodynamic therapy is difficult, however, in part because regional cerebral blood flow

or a surrogate marker of outcome has not been used to monitor efficacy. Two common inotropes used are dopamine and noradrenaline, both of which have direct cerebrovascular and cerebral metabolic effects in addition to their systemic hypertensive actions. Overly aggressive hemodynamic therapy carries the risk of adult respiratory distress syndrome. This study used positron emission tomography (PET) to assess the effects of the two commonly used inotropes for augmenting blood pressure, norepinephrine and dopamine, on cerebral blood flow (CBF).

Patients and Methods

The local research ethics committee approved the study. Twenty patients (13 female, 7 male; mean age 49 years, range 22–73 years) with proven aneurysmal SAH on digital subtraction angiography were studied in the environment of the neurosurgical neurointensive care unit and the Wolfson brain imaging center. World Federation of Neurological Surgeons grade[6] at presentation was grade 1 in one patient, grade 2 in four, grade 3 in six, grade 4 in five, and grade 5 in four. All patients received nimodipine and hemodynamic therapy including the use of dopamine or norepinephrine infusions guided by the use of a pulmonary arterial catheter. The target mean arterial blood pressure was in the range of 100 to 120 mmHg. Hydrocephalus was treated by external ventricular drainage, and a policy of early clipping of the ruptured aneurysm was employed. All grades of patient were considered eligible for this study if they were on or required an introduction of inotropic vasoactive agents to maintain mean arterial blood pressure within the target range or if they developed clinical evidence of ischemia possibly responsive to hypertensive therapy.

A General Electric Advance PET scanner was used together with the steady state intravenous $H_2{}^{15}O$ technique.[7] Serial blood gases were measured to ensure stability during the study. The median day of study was 4 days after SAH (range 1–22 days).

Results

Dopamine

There was an increase in mean arterial blood pressure in nine patients from 88 to 105 mmHg with no significant change in global CBF (overall change –0.6% ± 11%). To determine if there were any regional changes in distribution of CBF, the segmented PET brain images were used to generate frequency histogram data for each individual image. There was no statistical difference in the CBF distribution following the increase in mean arterial pressure with dopamine.

Norepinephrine

In the 11 patients receiving norepinephrine, mean arterial blood pressure increased from 94 to 113 mmHg but again there was no significant change in global CBF (overall change +6 ± 15%). There was a small but nonsignificant reduction of the number of voxels in the lower cerebral blood flow range and an increase in the number of voxels in the higher CBF range with the increase in blood pressure produced by norepinephine.

Discussion

This study suggests that in patients managed within a neurosurgical intensive care unit with mean arterial blood pressure within the region of 90 mmHg and with aggressive fluid management, further augmentation of blood pressure with the use of norepinephrine or dopamine confers no significant change in either global or regional CBF. There is no evidence of any significant redistribution of cerebral perfusion (intracerebral "steal"). Clinically this is important because it is the increase in mean arterial blood pressure beyond the target range of 90 mmHg that requires a higher dose of inotropes and that may render patients hemodynamically or cardiologically unstable. Although the whole population had no significant change in global or regional CBF, there were individual patients who did demonstrate large changes (both increases and decreases in CBF) when arterial blood pressure was increased. A larger study is now required to define whether there is a subgroup of patients whose global or regional CBF does increase with mean arterial blood pressure above 90 mmHg. It would appear that to minimize the risk of such supranormal levels of mean arterial pressure with additional inotropes, regional measurements of CBF should be performed to optimize the risk:benefit ratio.

Acknowledgment

Supported by a Medical Research Council Program Grant for the Study of Acute Brain Injury, a Clinical Research Training Fellowship to Pawan Minhas from the Royal College of Surgeons of England and the Medical Research Council (UK).

REFERENCES

1. Pickard ID, Matheson M, Patterson J, Wyper DJ. Prediction of late ischemic complications of cerebral aneurysm surgery by the intraoperative measurement of cerebral blood flow. J Neurosurg 1980;53:305–308

2. Pickard JD, Murray GD, Illingworth R, et al. Effect of oral nimodipine on cerebral infarction and outcome after subarachnoid haemorrhage: British aneurysm nimodipine trial. BMJ 1989;298: 636–642

3. Kassell NF, Peerless SJ, Durward QJ, et al. Treatment of ischemic deficits from vasospasm with intravascular volume expansion and induced arterial hypertension. Neurosurgery 1982;11: 337–343
4. Treggiari-Venzi MM, Suter PM, Romand JA. Review of medical prevention of vasospasm after aneurysmal subarachnoid haemorrhage: a problem of neurointensive care. Neurosurgery 2001; 48:249–261
5. Origitano TC, Wacher TM, Reichmann H, Anderson DE. Sustained increased cerebral blood flow with prophylactic hypertensive hypervolemic hemodilution ("triple-H" therapy) after subarachnoid hemorrhage. Neurosurgery 1990;27:729–740
6. Drake CG, Hunt WE, Sano K, et al. Report of World Federation of Neurological Surgeons Committee on a universal subarachnoid hemorrhage grading scale. J Neurosurg 1988;68:985–986
7. Minhas PS, Menon DK, Smielewski P, et al. Positron emission tomographic cerebral perfusion disturbances and transcranial Doppler findings among patients with neurological deterioration after subarachnoid hemorrhage. Neurosurgery 2003;52: 1017–1024

49

The Role of Isoprostane in Vasospasm After Aneurysmal Subarachnoid Hemorrhage in Humans: Preliminary Experience

MATTHIAS F. OERTEL, M.D., WOLFGANG DEINSBERGER, M.D., RODRIK BABAKHANLOU, M.SC, DIETER-KARSTEN BÖKER, M.D.

Abstract

Isoprostane (8-epi-prostaglandin $F2_{\alpha}$) is a product of the nonenzymatic, free radical–induced breakdown of arachidonic acid. It has been shown to possess vasoconstrictive properties in animal models, suggesting that it can lead to cerebral vasospasm. This study tested the hypothesis that isoprostane is linked to the pathogenesis of vasospasm after subarachnoid hemorrhage (SAH) in humans. A total of 21 studies were performed on five SAH patients (age 47 ± 5 years, Hunt and Hess grade 4). From day 1 to 10 after SAH sequential transcranial Doppler examinations were conducted. Vasospasm was diagnosed if the blood flow velocity of the middle cerebral artery was > 120 cm/sec, and the Lindegaard ratio was >3. Isoprostane was measured in arterial and jugular venous blood samples and cerebrospinal fluid. In addition, the arteriovenous difference for oxygen ($AVDO_2$) was evaluated to estimate cerebral metabolism. Vasospasm was found in 29% of studies. Middle cerebral artery flow velocity was significantly higher in studies with vasospasm (170 ± 40 cm/sec vs 96 ± 26 cm/sec, $p < .001$). On average, isoprostane concentrations were 166 ± 142 pg/L, 114 ± 69 pg/L and 64 ± 28 pg/L in arterial and jugular venous blood and cerebrospinal fluid, respectively. Mean $AVDO_2$ was 3.2 ±1.6 volume %. In studies with and without vasospasm, there was no difference in isoprostane concentrations in arterial or jugular venous blood or cerebrospinal fluid. In 71% of studies, isoprostane concentrations were higher in arterial than in jugular venous blood. No correlation was found between isoprostane concentration and $AVDO_2$. These preliminary results did not reveal an influence of isoprostane on cerebral vasospasm in humans. However, there is reason to speculate that the brain may take up isoprostane.

Isoprostane (8-epi-prostaglandin $F_{2\alpha}$) is a product of the nonenzymatic, free radical–induced breakdown of arachidonic acid. In neurological diseases with increased oxidative stress such as Alzheimer's disease, elevated concentrations of isoprostane have been found.[1] In localized cerebral ischemia, isoprostane has been isolated.[2] Isoprostane has vasoconstrictive properties and may contribute to cerebral vasospasm in animal models.[3] This study tested the hypothesis that isoprostane is linked to the pathogenesis of vasospasm

after subarachnoid hemorrhage (SAH) in humans. Additionally, cerebral metabolism was evaluated using the arteriovenous differences for oxygen, glucose, and lactate. It was hypothesized that isoprostane reduces cerebral metabolism due to vasospasm. We herein report preliminary results of our first patients.

Patients and Methods

A total of 21 studies were performed on five patients with SAH (mean age 47 ± 5 years, two males and three females, Hunt and Hess grade 4).[4] Within the first 10 days after SAH, sequential transcranial Doppler examinations were conducted with concomitant measurements of isoprostane and parameters of cerebral metabolism.

Isoprostane was analyzed from three sources: arterial and jugular venous blood and cerebrospinal fluid. All fluids were collected in 10 mL syringes containing ethylenedinitrilo tetra-acetic acid and butylated hydroxytoluene (0.002%). This antioxidant inhibits the formation of isoprostane in vitro by more than 90%. Samples were centrifuged at 4°C at 4000 rpm for 10 minutes, and the serum was removed and stored at –80°C until analysis. The analysis was performed using a commercially available enzyme-linked immunosorbent assay kit (Cayman Chemicals, Ann Arbor, MI, USA). Normal values for isoprostane in human plasma range from 5 to 40 pg/mL.

Cerebral metabolism was evaluated using arteriovenous differences (AVD) of glucose, oxygen (O_2), and lactate. Metabolic ratio was calculated as the ratio of $AVDO_2$ over AVD for glucose. A metabolic ratio of < 0.6 indicated an overuse of glucose relative to oxygen and is called relative hyperglycolysis. As previously reported in head injured patients, hyperglycolysis indicates that the brain is using anaerobic glycolysis instead of oxidative metabolism to generate energy.[5]

Cerebral vasospasm was diagnosed using transcranial Doppler (TCD) (Multidop 2, DWL, Ueberlingen, Germany) sonography. Mean flow velocities were measured in the middle cerebral artery and the extracranial internal carotid artery using transtemporal and submandibular approachs, respectively. Vasospasm was diagnosed if the middle cerebral artery mean flow velocity was > 120 cm/sec, and the Lindegaard ratio (ratio of flow velocity in middle cerebral artery to flow in extracranial internal carotid artery) was > 3.

Results

Vasospasm was found in 29% of studies. Middle cerebral artery mean flow velocity was significantly higher in studies with vasospasm (170 ± 40 cm/sec) compared with those without vasospasm (96 ± 26 cm/sec, $p < .001$). Mean isoprostane concentrations were 166 ± 142 pg/mL, 114 ± 69 pg/mL, and 64 ± 28 pg/mL in arterial and jugular venous blood and cerebrospinal fluid, respectively (Fig. 49–1). There were no significant differences in isoprostane concentrations in arterial and jugular venous blood and cerebrospinal fluid between patients with and without vasospasm (Fig. 49–2). In 71% of studies isoprostane concentrations were higher in arterial than in jugular venous blood, suggesting an uptake of isoprostane into the brain.

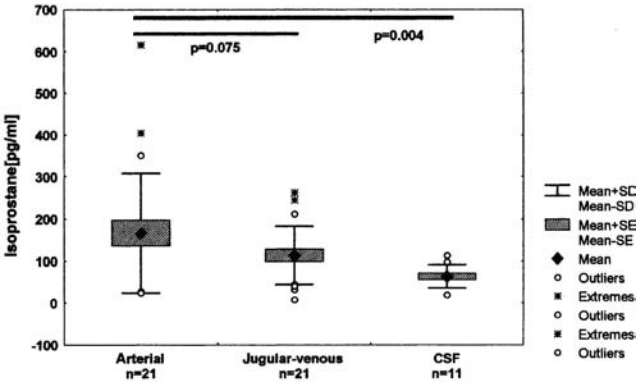


FIGURE 49–1 Isoprostane concentrations in arterial and jugular venous blood and cerebrospinal fluid. Arterial isoprostane concentrations were higher than jugular venous and cerebrospinal fluid concentrations. This may indicate that isoprostane is generated in organ systems other than the brain. Additionally, the brain takes up isoprostane.

Mean $AVDO_2$, AVDGlucose, and AVDLactate were 3.2 ± 1.6 volume %, 5.7 ± 7.4 mg %, and –0.36 ± 0.8 mg %,

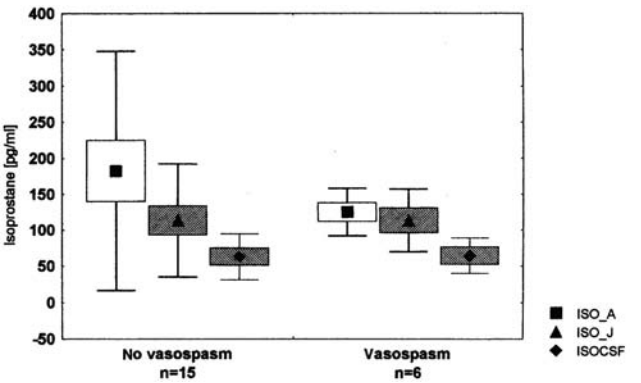


FIGURE 49–2 Isoprostane from arterial (ISO_A) and jugular venous blood (ISO_J) and cerebrospinal fluid (ISOCSF) in patients with and without vasospasm. Vasospasm was diagnosed using mean blood flow velocity in the middle cerebral artery and the Lindegaard ratio. There were no significant differences in isoprostane concentrations in arterial and jugular venous blood or cerebrospinal fluid in patients with and without vasospasm. Symbols are means, boxes are standard error of the mean, and bars are standard deviation.

TABLE 49–1 Pearson's Correlation Coefficients between Isoprostane Concentration from Three Sources and Transcranial Doppler and Metabolic Parameters

	Isoprostane Concentration		
	Arterial (N = 21)	**Jugular Venous (*N* == 21)**	**Cerebrospinal Fluid** (N = **11)**
Mean middle cerebral artery flow velocity	**–0.24**	**0.06**	0.10
$AVDO_2$	0.39 **(*p* =.08)**	**0.13**	0.09
AVDGlucose	**–0.02**	**0.22**	0.53 (*p* =.09)
AVDLactate	**–0.28**	–0.39 (*p*=.08)	–0.30

AVD, arteriovenous difference.

respectively. Lactate uptake into the brain was seen in 33% of studies. Relative hyperglycolysis was found in 53% of the studies. Lactate production was frequently seen in patients with relative hyperglycolysis. Isoprostane uptake was not dependent on the baseline metabolic situation. No statistical correlation was found between isoprostane and middle cerebral artery mean flow velocity, $AVDO_2$, AVDGlucose, and AVDLactate. However, some statistical trends ($p < .10$) were identified indicating an association of isoprostane with brain metabolism (Table 49–1).

Discussion

This chapter is based on preliminary data from an ongoing study at our institution. The role of isoprostane in cerebral vasospasm still needs to be defined. Isoprostane is a stable metabolite of free radical-related activity directed at arachidonic acid in biological membranes. Its biological half-life is about 1 minute.[6] Direct vasoconstrictive effects of isoprostane on isolated brain arterioles have been shown in animal models.[3] However, the data presented here do not show a difference in isoprostane concentrations between patients who developed vasospasm and those who did not. It should be noted that vasospasm was defined by TCD values and Lindegaard ratio. On average, plasma concentrations of isoprostane were higher in arterial than in jugular venous blood or cerebrospinal fluid. This indicates that isoprostane may be generated in organs other than the brain.

The most striking finding of the study is the uptake of isoprostane into the brain that occurred in the majority of patients in this study. This is an entirely new phenomenon in humans. Previous animal studies found that prostaglandin $F_{2\alpha}$ can cross the blood-brain barrier and exert effects on cerebral tissue perfusion.[7] In accordance with our results, it was speculated that despite its vasoconstrictive properties the primary action of systemically administered prostaglandin $F_{2\alpha}$ is on cerebral metabolism with only secondary changes in brain tissue perfusion.[8] It remains unclear whether and how isoprostane is metabolized by the brain thereafter.

The biochemical rationale behind the uptake of lactate after SAH also remains speculative. The phenomenon has been shown to occur in normal and pathological conditions.[9,10] Some authors concluded that the use of lactate reduced the consumption of glucose. Others found that there might be different compartments of energy production within the brain where glia cells generate lactate to feed neurons.[11] The overuse of glucose relative to oxygen may help to produce lactate in SAH patients. It also indicates that glycolysis increases, which may be explained by an increased need of energy in the form of adenosine triphosphate.

Conclusion

This work describes three new phenomena after SAH: uptake of lactate and isoprostane, the impact of isoprostane on cerebral metabolism, and hyperglycolysis. The implication for outcome of all three of these conditions will have to be clarified in the future.

REFERENCES

1. Montine TJ, Markesbery WR, Morrow JD, Roberts LJ II. Cerebrospinal fluid F2-isoprostane levels are increased in Alzheimer's disease. Ann Neurol 1998;44:410–413
2. Sakamoto H, Corcoran TB, Laffey JG, Shorten GD. Isoprostanes–markers of ischaemia reperfusion injury Eur J Anaesthesiol 2002;19:550–559
3. Sakamoto M, Takaki E, Yamashita K, et al. Nonenzymatic derived lipid peroxide, 8-iso-PGF2 alpha, participates in the pathogenesis of delayed cerebral vasospasm in a canine SAH model. Neurol Res 2002;24:301–306
4. Hunt WE, Hess RM. Surgical risk as related to time of intervention in the repair of intracranial aneurysms. J Neurosurg 1968;28: 14–20
5. Oertel M, Kelly DF, Lee JH, Glenn TC, Vespa PM, Martin NA. Metabolic suppressive therapy as a treatment for intracranial hypertension—why it works and when it fails. Acta Neurochir Suppl 2002;81:69–70
6. Morrow JD, Harris TM, Roberts LJ II. Noncyclooxygenase oxidative formation of a series of novel prostaglandins: analytical

ramifications for measurement of eicosanoids. Anal Biochem 1990;184:1–10
7. Pickard JD. Role of prostaglandins and arachidonic acid derivatives in the coupling of cerebral blood flow to cerebral metabolism. J Cereb Blood Flow Metab 1981;1:361–384
8. Pickard JD, MacDonell LA, Mackenzie ET, Harper AM. Prostaglandin-induced effects in the primate cerebral circulation. Eur J Pharmacol 1977;43:343–351
9. Chen T, Qian YZ, Di X, Zhu JP, Bullock R. Evidence for lactate uptake after rat fluid percussion brain injury. Acta Neurochir Suppl 2000;76:359–364
10. Smith D, Pernet A, Hallett WA, Bingham E, Masden PK, Amiel SA. Lactate: a preferred fuel for human brain metabolism in vivo. J Cereb Blood Flow Metab 2003;23:658–664
11. Magistretti PJ, Pellerin L, Rothman DL, Shulman RG. Energy on demand. Science 1999;283:496–497

50

S-100B and Neuron Specific Enolase as Predictors of Cerebral Vasospasm and Infarcts After Subarachnoid Hemorrhage

MATTHIAS F. OERTEL, M.D., UTE SCHUHMACHER, M.S., HEIKO MEWES, M.D., STEFANIE KÄSTNER, M.D., WOLFGANG DEINSBERGER, M.D., DIETER-KARSTEN BÖKER, M.D.

Abstract

S-100B and neuron specific enolase (NSE) are known to be good predictors of outcome in patients suffering from stroke and traumatic brain injury. However, their role in predicting outcome in patients after subarachnoid hemorrhage (SAH) is not clear. This study tested the hypothesis that S-100B and NSE measurements in the first 3 days after SAH can predict the development of both vasospasm and cerebral infarcts. Thirty-four patients with SAH (mean age: 54 ± 11 years, male:female ratio 1:1.4) were prospectively included in the study. S-100B and NSE were measured in mixed venous blood obtained from a central line upon arrival at the hospital and over the first 3 days after SAH. Vasospasm was diagnosed by serial transcranial Doppler sonographic studies. Vasospasm was defined as a mean flow velocity in the middle cerebral artery of > 120 cm/sec. The last cranial computed tomogram (CT) was assessed when the patients had their follow-up visits at a mean of 68 ± 104 days after SAH. Vasospasm was recorded in 44% of patients after SAH. S-100B values were significantly lower in patients who developed vasospasm than in those who did not (0.2 ± 0.2 µg/L vs 1.3 ± 1.7 pg/L, p = .03). Infarcts were seen in 40% of patients with vasospasm. NSE was significantly higher in patients who had infarcts on their last CT scans compared with patients who did not (20 ± 24 vs 8 ± 6 ng/L, p = .02). These results suggest that early measurements of S-100B and NSE may be good predictors of vasospasm and infarction on late CT scan in patients after SAH.

S-100B and neuron specific enolase (NSE) are known to predict outcome after traumatic brain injury and ischemic stroke. Elevated blood values of both S-100B and NSE have been associated with unfavorable clinical outcome and with large volume of cerebral infarction.[1–3] Despite numerous publications on traumatic brain injury and stroke, there is only limited information on S-100B and NSE in patients with subarachnoid hemorrhage (SAH). Most of these investigations evaluated S-100B and NSE at arbitrary time points after SAH in cerebrospinal fluid or plasma.[1,4] The preliminary results of these studies suggested that, in keeping with results in traumatic brain injury and ischemic stroke, S-100B and NSE may predict outcome. However, recent

investigators questioned the role of S-100B after SAH.[4] Cerebral vasospasm and subsequent infarction are known to be associated with unfavorable outcome after SAH. Elevation in serum levels of S-100B and NSE may occur as a consequence of vasospasm and cerebral infarction. However, it is unknown whether the S-100B and NSE levels immediately after the initial hemorrhage can predict the development of vasospasm and cerebral infarction.

This study tested the hypothesis that S-100B and NSE concentrations in blood taken within the first 3 days of SAH can predict the development of both vasospasm and infarction on cranial computed tomography (CT).

Patients and Methods

S-100B and NSE

S-100B and NSE were measured in mixed venous blood drawn from a central venous line. The samples were collected from the day of admission to day 3 after hemorrhage. In the cohort of 34 patients (mean age 54 ± 11 years, male:female ratio 1:1.4) a total of 61 measurements were performed. One, two, and three studies were conducted in 35, 50, and 15% of patients, respectively. On admission, the clinical condition of the patients were Hunt and Hess grade 1 in 18%, grade 2 in 24%, grade 3 in 32%, grade 4 in 20% and grade 5 in 6% of patients.[5] CT findings were Fisher grade 1 in 9%, grade 2 in 32%, grade 3 in 24%, and grade 4 in 35%.[6] S-100B and NSE assays were performed using a two-site radioimmunoassay technique (Byk Sangtec, Dietzenbach, Germany).

Normal values for S-100B were < .12 µg/L. S-100B concentrations were stratified into low (< 0.12 µg/L), intermediate (0.12–0.99 µg/L), and high values (> 1 µg/L). The rationale behind the cutoff for high S-100B concentration was the data of Raabe et al.[7] According to their receiver operating characteristic curve analysis, 1 µg/L provided an almost balanced sensitivity and specificity of around 60% for the prediction of poor outcome.

Normal values for NSE in adult patients are < 12.5 ng/L. According to our laboratory standards, pathological values were > 30 ng/L. Therefore, NSE data were stratified into normal (< 12.5 ng/L), intermediate (12.5–30 ng/L), and high values (> 30 ng/L).

Vasospasm and CT Scans

Serial transcranial Doppler (TCD) studies were conducted over the first 14 to 21 days following surgery. Vasospasm was diagnosed clinically in conjunction with TCD results. TCD criteria were a mean blood flow velocity in the middle cerebral artery of > 120 cm/sec. Serial cranial CT scans were obtained during hospital stay and follow-up. They were read by a neuroradiologist. The results of CT scans were compared over time, and whether a patient had a cerebral infarct related to the initial SAH was recorded.

Results

S-100B/NSE and Vasospasm

Vasospasm occurred during the observation period of up to 21 days posthemorrhage in 44% of patients (n = 15). Unfavorable outcome or death was seen in 60% of patients who experienced vasospasm. Mean serum S-100B values averaged over the first 3 days after hemorrhage were significantly lower in patients who later developed vasospasm (0.2 ± 0.2 µg/L vs 1.3 ± 1.7 pg/L, p = .03, Fig. 50–1). All initial parameters including NSE and the clinical condition of the patients were not significantly different between those who subsequently developed vasospasm and those who did not.

CT Findings and Their Relation to Early S-100B and NSE

CT scans showed infarcts in 32% of patients. Of those who developed vasospasm (n = 15), 40% of patients showed infarcts on their last CT scan. In patients with normal, intermediate, and high S-100B values, infarcts were seen in 11, 33, and 57% of patients, respectively (p = .15, χ-square, Fig. 50–2). Concentrations of NSE averaged over the first 3 days after SAH

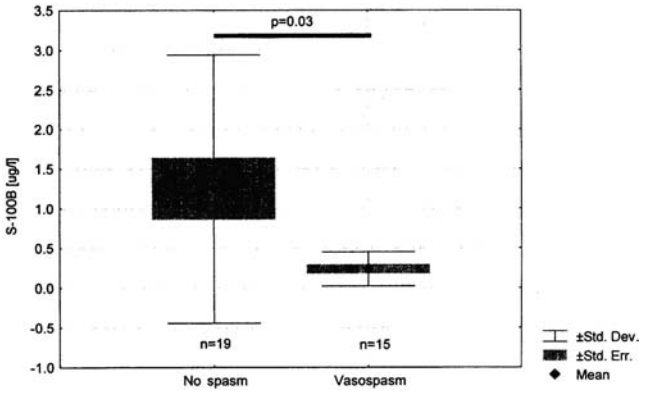


FIGURE 50–1 Mean S-100B concentrations in serum over the first 3 days after subarachnoid hemorrhage (SAH) in patients who developed vasospasm during their clinical course and those who did not. Boxes represent standard error of the mean (Std. Err.), and error bars are standard deviations (Std. Dev.). There was significantly less S-100B among patients who later developed vasospasm, suggesting that if the primary SAH caused a massive release of S-100B then vasospasm was unlikely to occur (p = .03).

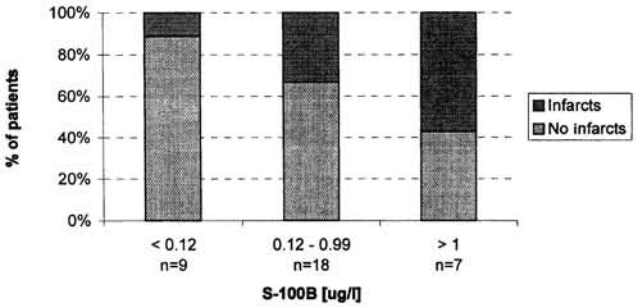


FIGURE 50–2 Frequency of cerebral infarction in last computed tomographic (CT) scan, by S-100B concentration. Infarcts were seen in increasing frequency as the S-100B concentration increased. There was a statistical trend but no significant relationship ($p = .15$, χ-square).

were significantly higher in patients who demonstrated infarcts on their last CT scans than in those who did not (20 ± 24 vs 8 ± 6 ng/L, $p = .02$, Students' t-test, Fig. 50–3).

Discussion

In this study vasospasm was defined by both blood flow velocity in the middle cerebral artery and Lindegaard ratio. In previous investigations predictors for the development of vasospasm were age < 50 years, good Hunt and Hess grade, Fisher grade 3 on initial CT scan, cigarette smoking, and hyperglycemia.[8] This study used clinical and radiological factors to predict vasospasm. Serum or other biochemical markers were not used to predict vasospasm.

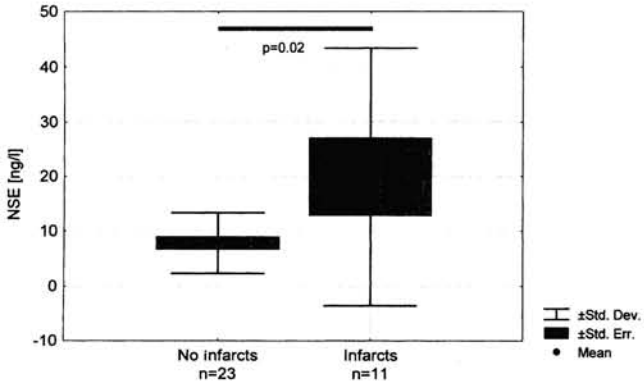


FIGURE 50–3 Mean neuron specific enolase (NSE) concentration in serum over the first 3 days after subarachnoid hemorrhage (SAH) in patients who developed cerebral infarction and those who did not. Boxes represent standard error of the mean (Std. Err.), and error bars are standard deviations (Std. Dev.). There was a significantly higher concentration of NSE in patients who developed infarction ($p = .03$, Student's t-test), suggesting that NSE is a good predictor of infarction after SAH.

This study notes that within the first 3 days after SAH, S-100B was significantly higher in patients who did not go on to experience vasospasm. Because S-100B is found mainly in glial cells, high concentrations of S-100B in mixed venous blood may indicate glial cell degradation. Among the many functions of glia, it has been suggested that glial cells provide energy to neurons.[9] Based on this assumption, one might speculate that damage to the glia could reduce neuronal energy supply and, as a result, cerebral metabolism might decrease. Regional cerebral blood flow would also be expected to decrease and cerebral blood flow velocities might remain low. We examined S-100B and NSE within the first 3 days of SAH. After the onset of vasospasm and in cases of secondary brain damage due to either or both SAH and vasospasm, S-100B and NSE might increase as a result of these processes.[10]

Cerebral infarcts are associated with glial and neuronal cell death. The latter can be accompanied by increased NSE concentrations.[2,3] In the present study, NSE correlated significantly with S-100B. This may indicate that the release of NSE and that of S-100B are linked over time. The time course of change in S-100B may be different in patients with traumatic brain injury and SAH compared with those with ischemic stroke. In ischemic stroke, a gradual release in both proteins has been shown over the first 3 days.[3] In traumatic brain injury and SAH, S-100B and NSE have been shown to be elevated within the initial phase depending on the amount of injury. This increase was followed by a decrease and then a second peak at later time points.[10] The second increase has been suggested to be due to secondary brain injury.

Conclusion

This study suggests that serum S-100B and NSE values obtained within the first 3 days of SAH are valuable markers for prediction of whether a patient will develop vasospasm and cerebral infarction in CT scan. S-100B is a reliable predictor of outcome and the development of vasospasm, whereas NSE is linked to the development of cerebral infarcts after SAH. Patients with high S-100B are unlikely to develop vasospasm.

REFERENCES

1. Persson L, Hardemark H, Edner G, Ronne E, Mendel-Hartvig I, Pahlman S. S-100 protein in cerebrospinal fluid of patients with subarachnoid haemorrhage: a potential marker of brain damage. Acta Neurochir (Wien) 1988;93:116–122
2. Cunningham RT, Young IS, Winder J, et al. Serum neuron specific enolase (NSE) levels as an indicator of neuronal damage in patients with cerebral infarction. Eur J Clin Invest 1991;21: 497–500

3. Missler U, Wiesmann M, Friedrich C, Kaps M. S-100 protein and neuron-specific enolase concentrations in blood as indicators of infarction volume and prognosis in acute ischemic stroke. Stroke 1997;28:1956–1960
4. Kay A, Petzold A, Kerr M, Keir G, Thompson E, Nicoll J. Decreased cerebrospinal fluid apolipoprotein E after subarachnoid hemorrhage: correlation with injury severity and clinical outcome. Stroke 2003;34:637–642
5. Hunt WE, Hess RM. Surgical risk as related to time of intervention in the repair of intracranial aneurysms. J Neurosurg 1968;28:14-20
6. Fisher CM, Kistler JP, Davis JM. Relation of cerebral vasospasm to subarachnoid hemorrhage visualized by computerized tomographic scanning. Neurosurgery 1980;6:1–9
7. Raabe A, Grolms C, Seifert V. Serum markers of brain damage and outcome prediction in patients after severe head injury. Br J Neurosurg 1999;13:56–59
8. Charpentier C, Audibert G, Guillemin F, et al. Multivariate analysis of predictors of cerebral vasospasm occurrence after aneurysmal subarachnoid hemorrhage. Stroke 1999;30:1402–1408
9. Magistretti PJ, Pellerin L. Cellular mechanisms of brain energy metabolism and their relevance to functional brain imaging. Philos Trans R Soc Lond B Biol Sci 1999;354:1155–1163
10. Takayasu M, Shibuya M, Kanamori M, et al. S-100 protein and calmodulin levels in cerebrospinal fluid after subarachnoid hemorrhage. J Neurosurg 1985;63:417–420

51

The APACHE Score for Early Detection of Cerebral Vasospasm

YASUHIKO HAYASHI, M.D., HIROSHI SHIMA, M.D.,
YOSHIKAZU GOTO, M.D., SHINYA KIDA, M.D.,
HIDEO INABA, M.D., JUNKOH YAMASHITA, M.D., PH.D.

Abstract

There has been progress with regard to the diagnosis and treatment of vasospasm although the prediction of which patients will develop vasospasm remains difficult. Subarachnoid hemorrhage (SAH) due to a ruptured aneurysm and the subsequent development of cerebral vasospasm represents a considerable systemic stress to the human organism. There are few reports, however, that investigate physiological indices in the patient, as a measure of the general effect of SAH and vasospasm, to determine the correlation of these indices with subsequent vasospasm. One such index is the acute physiological and chronic health evaluation (APACHE) score. This report analyzed the ability of the APACHE score to detect vasospasm and to discriminate effects of treatment in patients with SAH. Forty-four patients with SAH due to ruptured aneurysm were evaluated. There were 13 men and 31 women with a mean age of 68 years. Data were collected to calculate the systemic inflammatory response syndrome (SIRS) and the acute physiology (AP) score components of APACHE II and III. Patients were classified by their Hunt and Kosnik grade (1–3) and by the type of vasospasm they developed (none, asymptomatic, or symptomatic). Scores were collected on the day of SAH and on days 1, 3, 5, 7, and 9. Patients treated with barbiturate coma or mild hypothermia were also evaluated. The maximum scores for the SIRS was 1.5 for patients with no vasospasm, 2.3 for asymptomatic, and 3.0 for symptomatic vasospasm. Corresponding maximum scores for APACHE II were 3.4, 6.0, and 9.0 and for APACHE III, 11.1, 22.0, and 29.3, respectively. All of the scores were elevated with the development of vasospasm and with symptoms due to vasospasm. APACHE III showed the most marked increase and this was attributed to tachycardia, tachypnea, acid-base imbalance, and hyponatremia. In patients with symptomatic vasospasm, the APACHE III score increased before the onset of symptoms from vasospasm. This increase in APACHE III score was detectable even in patients treated with barbiturate coma or mild hypothermia. The effect of vasospasm as measured on the APACHE III scale was similar to the effect of a primary Hunt and Kosnik grade 4 SAH. These results suggest that the APACHE III score is a useful measurement to use for the early detection of vasospasm and to evaluate the effectiveness of treatment. It tends to increase during the onset of vasospasm before symptoms develop, suggesting that careful observation is essential to detect and treat symptomatic vasospasm immediately. The score was useful even if clinical neurological examination was difficult because of barbiturate coma or mild hypothermia.

Aneurysmal subarachnoid hemorrhage (SAH) and the subsequent development of cerebral vasospasm is a substantial physiological stress to the patient. Few reports, however, have investigated the occurrence of vasospasm from the standpoint of general physiological changes. Some attention has been paid to systemic inflammatory markers and to changes in the cerebrospinal fluid and their relation to vasospasm.[1,2] Indeed, vasospasm has been associated with and attributed in part to elevation of inflammatory cytokines in the cerebrospinal fluid. A role for inflammation is also suggested by histological studies showing inflammatory cells in the walls of vasospastic arteries.[3–5] Several of these reports note that inflammatory cytokines are markedly elevated coincident with the development of vasospasm and, furthermore, that the degree of elevation is correlated with the severity of vasospasm.

The revised acute physiology and chronic health evaluation (APACHE) II, which has 12 physiological variables including the Glasgow coma scale, has frequently been applied to patients in intensive care units to evaluate the severity or prognosis of various diseases.[6] The APACHE III, which is a newer revised system, includes improved incorporation of physiological measures, chronic health measures, and disease classification as well as an examination of the susceptibility of the measures to various measurement biases.[7] This study used the acute physiology (AP) scores in the APACHE system to determine whether this could be used to predict the development of vasospasm and to gain some insight into the physiological effects of SAH and vasospasm. The AP scores include all of the measures of the APACHE score except the Glasgow coma score and the disease classification.

Materials and Methods

Studies were performed on 44 patients, age 38 to 89 years, with Hunt and Kosnik grade 1 to 3 aneurysmal SAH.[8] Patients were admitted within 48 hours of SAH. Hunt and Kosnik grades were 1 in four patients (9%), 2 in 19 (44%), and 3 in 21 (47%). The Fisher scores based on admission cranial computed tomography (CT) were 2 in seven (16%), 3 in 32 (72%), and 4 in five (12%).[9] The aneurysm was clipped in 23 patients (52%), coiled in 17 (39%), and treated conservatively in four (9%). Patients were divided into three groups according to their status with regard to vasospasm. There were 28 patients (64%) that did not develop clinical or radiological evidence of vasospasm. Eight (18%) had asymptomatic vasospasm as judged by radiological evidence of vasospasm in the absence of either or both clinical symptoms and signs. The third group included eight patients (18%) with both clinical signs and radiological evidence of vasospasm (symptomatic vasospasm). Radiological evidence of vasospasm included low-density areas on CT scan, hypoperfusion on cerebral blood flow measurement, velocity > 120 cm/sec, or an increase of > 20 cm/sec/day on transcranial Doppler sonography and/or narrowing of arteries on cerebral angiography. Patients with severe systemic complications and Hunt and Kosnik grades 4 and 5 were excluded.

The scoring systems used were systemic inflammatory response syndrome (SIRS), APACHE II, and APACHE III. Parameters of SIRS are heart rate, body temperature, respiratory rate, and the white blood cell count. APACHE II includes heart rate, body temperature, blood pressure, respiratory rate, and laboratory data (white blood count, pO_2, pH, and serum Na+, K+, creatinine, and hematocrit).[6] APACHE III adds blood urea nitrogen, bilirubin, albumin, blood sugar, and urine volume and removes serum K+ from APACHE II. The sum of each score is 4, 45, and 204, respectively.[7] Each score was recorded on days 0 to 9 post-SAH. All statistical comparisons were made by Student's *t*-test.

Results

The maximum points for each score that was recorded between days 5 and 9 were compared for the three groups. The maximum scores for the SIRS was 1.5 for patients with no vasospasm, 2.3 for asymptomatic, and 3.0 for symptomatic vasospasm. Corresponding maximum scores for APACHE II were 3.4,6.0, and 9.0 and for APACHE III, 11.1,22.0, and 29.3, respectively (Fig. 51–1). For the SIRS there was a statistically significant

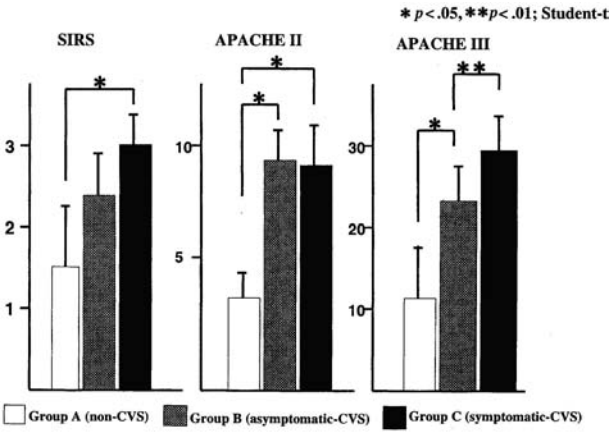


FIGURE 51–1 The systemic inflammatory response syndrome (SIRS), acute physiological and chronic health evaluation (APACHE II), and APACHE III scores for patients who did not develop vasospasm (group A), patients with asymptomatic vasospasm (group B), and patients with symptomatic vasospasm (group C). There is a progressive increase in score with development of vasospasm and with symptoms from vasospasm, and the APACHE III score discriminates most significantly between the three groups.

difference in score between patients with no vasospasm and those with symptomatic vasospasm. For APACHE II, there was, in addition to significant differences between those groups, a significant difference between patients with no vasospasm and those with asymptomatic vasospasm. The APACHE III score demonstrated significant differences between all three groups. The parameters of APACHE III that contributed most to the elevations were heart rate, blood pressure, respiratory rate, pH, and serum Na+.

The time course of the AP components of each of the scoring systems was compared (Fig. 51–2). The SIRS was statistically different only by comparison of values 4 days before the onset of vasospasm with the day of vasospasm in patients with symptomatic vasospasm. The APACHE II was significantly different between the day of vasospasm and the score 2 and 4 days earlier in patients with symptomatic vasospasm. For APACHE III there was more discrimination in scores in that there was a significant difference in the score between the day of vasospasm and 2 and 4 days earlier and between the scores 2 and 4 days before vasospasm in patients with symptomatic vasospasm. In addition, in patients with asymptomatic vasospasm the APACHE III score showed the same significant differences as in patients with symptomatic vasospasm.

The APACHE III was examined in three patients who had barbiturate coma therapy and three patients

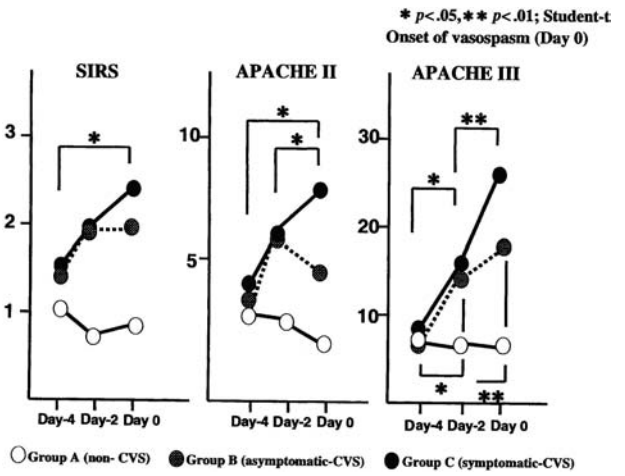


FIGURE 51–2 The time course of scores on the three scales for patients who did not develop vasospasm (group A), patients with asymptomatic vasospasm (group B), and patients with symptomatic vasospasm (group C). Day 0 indicates the day of vasospasm, day-4 is 4 days before onset of vasospasm, and day-2 is 2 days before vasospasm. There is a progressive increase in score with time in patients with asymptomatic and symptomatic vasospasm, and the acute physiological and chronic health evaluation (APACHE) III score discriminates most significantly between the three groups.

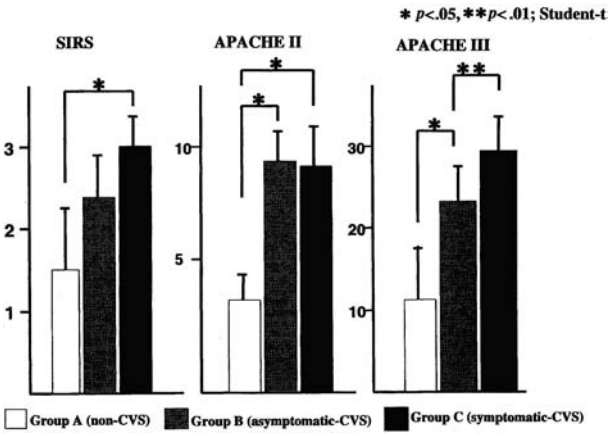


FIGURE 51–3 Scores on the acute physiology (AP) component of the acute physiological and chronic health evaluation (APACHE) III scale with patients graded according to the five Hunt and Kosnik clinical grades on the *x*-axis.[8] Scores on the AP component of the APACHE III scale for patients who did not develop vasospasm (group A), patients with asymptomatic vasospasm (group B), and patients with symptomatic vasospasm (group C). The score increases with increasing neurological grade and with asymptomatic and symptomatic vasospasm.

treated with hypothermia. In three of these six patients, the APACHE III elevated with the occurrence of asymptomatic vasospasm. In the remaining three patients there was no radiological evidence of vasospasm and no elevation of the APACHE III score was noted. None of the six patients developed severe general complications.

The APACHE III score at the onset of SAH was compared with that during vasospasm (Fig. 51–3). APACHE II scores were collected in patients with all five Hunt and Kosnik grades. Patients with grades 1 to 3 showed no elevation of scores, and the values were similar to those of patients who did not develop vasospasm. Hunt and Kosnik grade 4 patients had moderately elevate scores that were similar to those of patients with asymptomatic vasospasm. The grade 5 patients showed remarkably elevated scores that were even greater than those of patients with symptomatic vasospasm.

Discussion

This study used the APACHE system to evaluate the general systemic response to SAH and to elucidate the physiological changes that occur in response to cerebral vasospasm.[6,7] The results suggest that the SIRS and APACHE II are not of much value in the detection of vasospasm prior to the occurrence of symptoms but that the APACHE III may be of some use in terms of predicting the onset of symptomatic and asymptomatic

vasospasm. In the APACHE III score, changes in heart rate, blood pressure, respiratory rate, pH, creatinine, and serum Na^+ were most remarkable. Tachycardia and hypertension seem to originate from changes in sympathetic tone or as a physiological compensation to the increase in cardiac output. Tachypnea is assumed to occur because of ischemic changes in the upper brain stem, and acid–base imbalance seems to occur with tachypnea. Hyponatremia is considered to arise as the result of salt-wasting syndrome, or the retention of water occurred due to the syndrome of inappropriate secretion of antidiuretic hormone.[1,2]

APACHE III scores were noted to increase in cases of asymptomatic vasospasm and to further increase prior to the onset of symptoms from vasospasm. These predictive abilities were not noted for SIRS and APACHE II. These results strongly suggest that APACHE III is effective for the early detection of vasospasm. If the scores of APACHE III begin to rise in a patient with aneurysmal SAH, it may be necessary to observe more carefully whether the scores continue to rise and to consider tests for the early detection of vasospasm.

Detection of vasospasm in patients who are in barbiturate coma or who are treated with hypothermia can be very difficult. It is well known that barbiturate or hypothermia therapy can be associated with general complications such as renal failure, infections, electrolyte abnormality, and cardiac arrhythmias. The present series contains only three cases with vasospasm and three without vasospasm who were treated with barbiturate coma or hypothermia. In this small number of patients, however, an increase in the APACHE III score was noted with the development of vasospasm. These preliminary results suggest that the APACHE III score may be of value even in these cases.

Finally, we used the APACHE III score as a surrogate marker of the general invasiveness or physiological stress associated with SAH and vasospasm. Asymptomatic vasospasm was associated with an APACHE III score similar to that of a Hunt and Kosnik grade 4 SAH and symptomatic vasospasm was intermediate between grade 4 and 5. As expected, vasospasm seems to cause considerable stress akin to that of a severe SAH.

Conclusions

APACHE III may be a valid index for the early detection of cerebral vasospasm and the evaluation of effectiveness of treatment. It may be useful even in patients who are difficult to evaluate clinically because of barbiturate coma or hypothermia.

REFERENCES

1. Dumont AS, Dumont RJ, Chow MM, et al. Cerebral vasospasm after subarachnoid hemorrhage: putative role of inflammation. Neurosurgery 2003;53:123–135
2. Sercombe R, Dinh YR, Gomis P. Cerebrovascular inflammation following subarachnoid hemorrhage. Jpn J Pharmacol 2002;88: 227–249
3. Fassbender K, Hodapp B, Rossol S, et al. Inflammatory cytokines in subarachnoid hemorrhage: association with abnormal blood flow velocities in basal arteries. J Neurol Neurosurg Psychiatry 2001;70:534–537
4. Kikuchi T, Okuda Y, Kaito N, et al. Cytokine production in cerebrospinal fluid after subarachnoid hemorrhage. Neurol Res 1995;17:106–108
5. Gaetani P, Tartara F, Pignatti P, et al. Cisternal CSF levels of cytokines after subarachnoid hemorrhage. Neurol Res 1998;20: 337–342
6. Knaus WA, Draper EA, Wagner DP, et al. APACHE II: a severity of disease classification system. Crit Care Med 1985;13:818–829
7. Wagner D, Draper E, Knaus W. APACHE III study design: development of APACHE III. Crit Care Med 1989;17:S199–S203
8. Hunt WE, Kosnik JE. Timing and perioperative care in intracranial aneurysm surgery. Clin Neurosurg 1974;21:79–89
9. Fisher CM, Kistler JP, Davis JM. Relation of cerebral vasospasm to subarachnoid hemorrhage visualized by computerized tomographic scanning. Neurosurgery 1980;6:1–9

52

Sequential Changes in Oxyhemoglobin in Fluid Drained from SAH Patients Undergoing Cisternal Irrigation

TAKU SATO, M.D., HIROYUKI MURAMATSU, M.D., MASAHIRO OINUMA, M.D., YUTAKA KONNO, M.D., KYOUICHI SUZUKI, M.D., MASATO MATSUMOTO, M.D., TATSUYA SASAKI, M.D., NAMIO KODAMA, M.D.

Abstract

Cisternal irrigation therapy with urokinase and ascorbic acid has been performed to prevent vasospasm after aneurysmal subarachnoid hemorrhage (SAH). Urokinase is used to dissolve the residual clot and ascorbic acid is added to break down the oxyhemoglobin in the clot that is thought to contribute to the development of vasospasm. Cisternal irrigation therapy has been used in 310 patients. The present study evaluated the effect of ascorbic acid by measuring oxyhemoglobin in fluid collected from 87 patients in whom cisternal irrigation with urokinase and ascorbic acid was performed. The concentration of oxyhemoglobin in the drainage fluid from these patients was compared with that obtained from 37 patients who underwent cisternal drainage only, without irrigation. The absorption spectrum of the drained fluid was measured daily, and the sequential changes of the oxyhemoglobin specific 576 nm peak in both groups were compared. In the irrigation group, the mean value of the oxyhemoglobin specific peak decreased over time. Four patients with massive intracerebral hematomas on admission computed tomography showed an atypical pattern of continuous high levels of oxyhemoglobin in the drainage fluid. In five patients who developed vasospasm, the mean value of oxyhemoglobin was higher than the value in the 82 patients without vasospasm. In patients undergoing only cisternal drainage, the oxyhemoglobin peak decreased in the early stage, but the values increased again and remained high in most cases until day 12. Oxyhemoglobin concentrations in fluid from patients undergoing irrigation with urokinase and ascorbic acid were lower than those of the drainage group. These results suggest that ascorbic acid is effective in converting oxyhemoglobin into nonspasmogenic substances. Ascorbic acid may play a role in prevention of symptomatic vasospasm.

Cerebral vasospasm is one of the most serious complications of aneurysmal subarachnoid hemorrhage (SAH). Because definitive treatments for symptomatic vasospasm have not been established, methods aimed at their prevention predominate. We performed cisternal irrigation with urokinase and ascorbic acid to prevent the occurrence of symptomatic vasospasm after aneurysmal SAH.[1,2] Urokinase was used to dissolve the residual clot and ascorbic acid[3,4] was included to convert spasmogenic oxyhemoglobin[5] into nonspasmogenic substances.

We evaluated the effect of ascorbic acid by measuring oxyhemoglobin in fluid drained from patients undergoing cisternal irrigation and compared the results to those obtained in patients undergoing only cisternal drainage. In addition, changes in patients with symptomatic vasospasm are described.

Materials and Methods

Since April 1984, 310 patients with aneurysmal SAH underwent cisternal irrigation at our institute. The present study included 124 patients of which 87 (70%, irrigation group) received cisternal irrigation with urokinase and ascorbic acid and 37 (30%) were treated with cisternal drainage alone (drainage group). Cisternal irrigation was chosen when the preoperative computed tomogram (CT) showed hemorrhage with a CT (Hounsfield) number > 60,[6] indicating a significant risk for symptomatic vasospasm. Cisternal drainage alone was selected when the preoperative CT scans showed the SAH was Fisher group 2 or 3,[7] and the highest CT number of the clot was < 60. All patients underwent surgery within 72 hours of SAH. In the irrigation group, a drainage tube was placed in the prepontine or chiasmatic cistern, and an infusion tube was inserted into the sylvian fissure. Lactated Ringer's solution without urokinase, but with ascorbic acid (4 mg/mL), was infused for the first 12 hours after surgery Thereafter, urokinase (120 IU/mL) was added in the irrigation solution. Urokinase administration was delayed to reduce the risk of postoperative hemorrhage. The irrigation fluid was adjusted to the same pH (7.2–7.6) and osmotic pressure (280–300 mOsm/kg) as normal cerebrospinal fluid. The infusion rate was 30 mL/hour. The mean duration of irrigation was 10 days.

The effect of ascorbic acid was examined by measuring the concentration of oxyhemoglobin every day in the drained fluid of the two groups. Oxyhemoglobin was assessed spectrophotometrically by sequential changes in absorption at 576 nm, which is specific for oxyhemoglobin.

Results

In patients undergoing cisternal irrigation with urokinase and ascorbic acid, oxyhemoglobin decreased continuously for the first 5 days after SAH and then remained detectable at only low concentrations (Fig. 52–1). On the other hand, in the nonirrigated patients treated with drainage alone, oxyhemoglobin concentration declined until 3 days after SAH and then increased again, reaching the highest value on day 9. Values remained higher than in the irrigation group throughout the 13-day observation period. Five patients undergoing irrigation therapy (6%) developed symptomatic vasospasm, whereas none of the patients treated with drainage alone developed symptomatic vasospasm.

Among the five patients with vasospasm, one developed transient and four had permanent hemiparesis. The mean values of the absorption spectrum of oxyhemoglobin in these five patients were higher than in the 82 irrigated patients without vasospasm. However, the difference did not reach statistical significance (Fig. 52–2). The oxyhemoglobin absorption spectrum of three of the five patients with vasospasm increased after day 4 and remained high. In the two remaining patients, it remained low (Fig. 52–3). One of these patients underwent hemodialysis and developed status epilepticus due to difficulties in managing the water balance. The other patient had severe diabetes mellitus and a unilateral internal

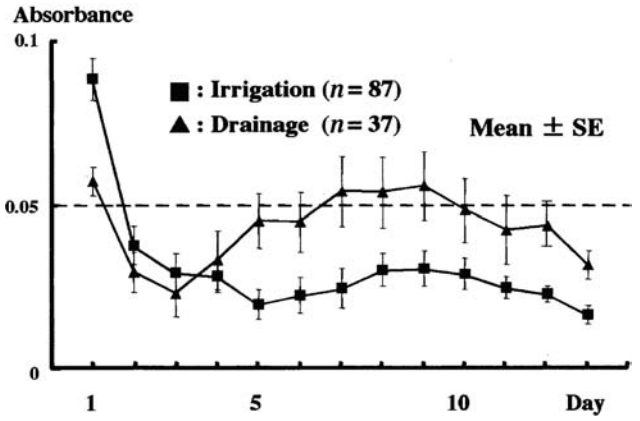


FIGURE 52–1 Spectrometric assessment of sequential changes in the mean height of the oxyhemoglobin peak (values are means ± standard error of the mean [SE]). The peak due to oxyhemoglobin (576 nm) decreased by 5 days after subarachnoid hemorrhage and remained low in the group undergoing cisternal irrigation (irrigation). In contrast, in patients treated by drainage alone (drainage), it increased after day 3, reached its highest value on day 9, and remained higher than in the irrigated group until day 13.

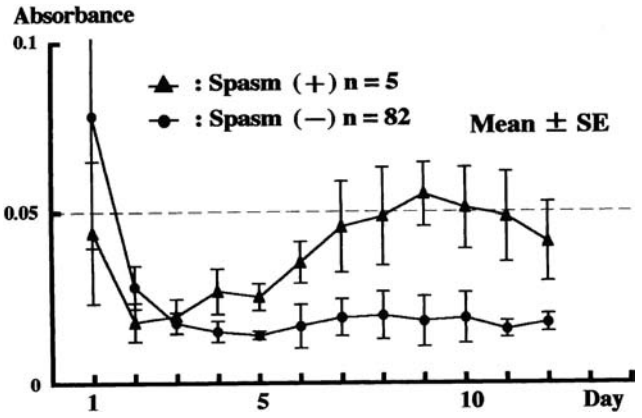


FIGURE 52–2 Graph showing sequential changes in the absorption spectrum of oxyhemoglobin, based on the spectrophotometric peak at 576 nm. Values are means ± standard error of the mean (SE). In patients who developed symptomatic vasospasm the mean value increased gradually after a transient decrease. On the other hand, in patients without vasospasm the initial decline in oxyhemoglobin was not followed by an increase.

carotid artery occlusion prior to SAH. She developed symptomatic vasospasm without an increase in the absorption spectrum.

Discussion

In experimental studies, ascorbic acid was shown to convert oxyhemoglobin into reduced hemoglobin and finally into verdoheme-like, nonspasmogenic products.[3,4] The present study demonstrates the effect of ascorbic acid in patients with SAH. Whereas cisternal irrigation with ascorbic acid resulted in a decrease in the oxyhemoglobin level in drained fluid, cisternal drainage alone did not.[8] These observations confirm that in both the clinical and the experimental settings, oxyhemoglobin can be broken down by ascorbic acid. Because the volume of the primary SAH was different in our two groups, it is not valid simply to compare them with respect to the results of their treatments.

Sequential changes in the mean values of the absorption spectrum revealed that the oxyhemoglobin-specific peak decreased by day 5 and remained low in irrigated patients. In the five patients with vasospasm, it increased after a transient decline. The peak height, however, was not significantly different from patients without vasospasm. In fact, the spectrum in two of the patients with vasospasm remained low throughout the 12-day observation period. The relationship between symptomatic vasospasm and the absorption spectrum of oxyhemoglobin in drained fluid remains unclear. Further investigations are under way in our laboratory to elucidate the role of oxyhemoglobin in cerebral vasospasm.

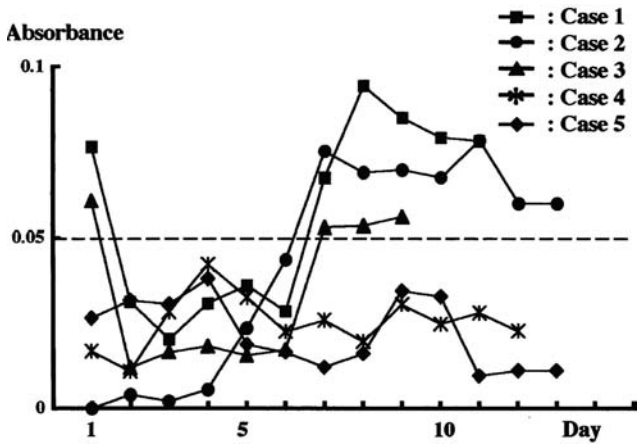


FIGURE 52–3 Graph showing sequential changes in the oxyhemoglobin absorption peak in five patients who developed symptomatic vasospasm. In three of the five patients, the absorption spectrum increased starting on approximately day 5 after subarachnoid hemorrhage and remained high thereafter. In contrast, in two patients the oxyhemoglobin absorption was as low as in patients without symptomatic vasospasm.

Conclusion

We sequentially assayed the oxyhemoglobin concentration in fluid drained from patients undergoing cisternal irrigation with urokinase and ascorbic acid and compared the values to patients undergoing cisternal drainage only. The oxyhemoglobin concentration was lower in the group undergoing cisternal irrigation with urokinase and ascorbic acid than in patients who received cisternal drainage alone. Our results support earlier reports that ascorbic acid effectively converts oxyhemoglobin into nonspasmogenic substances. The mean values of the absorption spectrum were higher in five patients who developed vasospasm compared with the 82 patients who did not. Investigations are under way to identify the role of oxyhemoglobin in cerebral vasospasm.

REFERENCES

1. Kodama N, Sasaki T, Kawakami M, Sato M, Asari J. Cisternal irrigation therapy with urokinase and ascorbic acid for prevention of vasospasm after aneurysmal subarachnoid hemorrhage: outcome in 217 patients. Surg Neurol 2000;53:110–118

2. Sasaki T, Kodama N, Kawakami M, et al. Urokinase cisternal irrigation therapy for prevention of symptomatic vasospasm after aneurysmal subarachnoid hemorrhage: a study of urokinase concentration and the fibrinolytic system. Stroke 2000;31: 1256–1262

3. Kawakami M, Kodama N, Toda N. Suppression of the cerebral vasospastic actions of oxyhemoglobin by ascorbic acid. Neurosurgery 1991;28:33–40

4. Sato M. Prevention of cerebral vasospasm: experimental studies on the degradation of oxyhemoglobin by ascorbic acid. Fukushima J Med Sci 1987;33:55–70
5. Osaka K. Prolonged vasospasm produced by the break-down products of erythrocytes. J Neurosurg 1977;47:403–411
6. Suzuki J, Komatsu S, Sato T, Sakurai Y. Correlation between CT findings and subsequent development of cerebral infarction due to vasospasm in subarachnoid hemorrhage. Acta Neurochir (Wien) 1980;55:63–70
7. Fisher CM, Kistler JR, Davis JM. Relation of cerebral vasospasm to subarachnoid hemorrhage visualized by computerized tomographic scanning. Neurosurgery 1980;6:1–9
8. Konno Y, Sato T, Suzuki K, Matsumoto M, Sasaki T, Kodama N. Sequential changes of oxyhemoglobin in drained fluid of cisternal irrigation therapy-reference to the effect of ascorbic acid. Acta Neurochir Suppl 2001;77:167–169

53

Sex and Racial Factors in the Outcome of Subarachnoid Hemorrhage in Mississippi

DONNIE R. TYLER II, M.D., VIRGINIA L. OVERMAN, M.H.S.,
EDWARD R. FLOTTE, M.D., JOHN D. TYLER, B.S.,
WILLIAM D. JOHNSON, PH.D., JOHN H. ZHANG, M.D., PH.D.

Abstract

This work evaluated the prognostic factors for outcome in patients with aneurysmal subarachnoid hemorrhage (SAH) and studied the effect of race and sex on outcome. Medical records from 96 patients seen at the University of Mississippi Medical Center between the autumn of 1993 and the spring of 1999 were reviewed. All patients presented to the emergency room with computed tomographically (CT)-verified SAH and arteriogram-documented anterior circulation aneurysms. Multiple factors were analyzed for each patient, including Hunt and Hess grade, age, race, sex, comorbid conditions, aneurysm location, elevated large vessel velocities by transcranial Doppler, and level of function at discharge. The development of increased transcranial velocities by Doppler was close to 67% in all patient populations. Clinical signs of vasospasm were present in 38 to 53% of the population. Overall mortality rates were 46% for African American males, 36% for Caucasian females, 20% for Caucasian males, and 25% for African American females. A large percent (78%) of our patient population had one or more comorbid factors such as hypertension, diabetes, morbid obesity, or cardiovascular disease. We found that the presenting modified Hunt and Hess grade correlated with gross functional outcome. Factors correlating with poor outcome were male sex, presence of concomitant health illnesses, and clinical evidence of vasospasm.

Mississippi is located in the "black-belt" of the southern United States, a region with a significantly higher rate of obesity, diabetes, hypertension, cardiac, and cerebral vascular diseases than in other parts of the nation. The correlation of features of aneurysmal subarachnoid hemorrhage (SAH), including survival, with patient gender and race and comorbid health problems has not been studied in Mississippi prior to this retrospective chart review.[1,2]

Materials and Methods

Patients

A retrospective analysis of all patients presenting to the University of Mississippi emergency room with a computed tomographically (CT)-diagnosed SAH and subsequent arteriogram-verified anterior circulation aneurysm was performed for patients seen between the autumn of 1993 and the spring of 1999. Ninety-six

patients were identified. The cohort sex and racial distribution was similar to that of the University of Mississippi patient base. The adult patient distribution at the University of Mississippi is 39% African American female, 27% African American male, 17% Caucasian female, and 17% Caucasian male. The study population was composed of 42% African American females, 20% African American males, 20% Caucasian females, and 18% Caucasian males. The differences in the sex and race distributions between these two patient populations are not statistically significant (χ-square = 2.7, p = .5). Data collected on each patient included presenting clinical neurological status, age, race, sex, comorbid health conditions, aneurysm location, elevated large vessel velocities by transcranial Doppler, development of clinically evident vasospasm, the level of function at discharge, and discharge destination.[3,4] Relevant aspects of past medical history analyzed included diabetes, hypertension, chronic obstructive pulmonary disease, morbid obesity, and congestive heart failure.

Treatment

All patients were monitored under similar conditions during their stay at the University of Mississippi Medical Center. The need for intubation and other supportive measures was determined based on the patient's clinical status.[5] Emergent CT scan of the head and basic laboratory studies were obtained. If indicated, an external ventricular drain was placed in the emergency room under sterile conditions. A four-vessel cerebral arteriogram was then performed. Each patient was admitted to the intensive care unit for close observation. Surgical aneurysm clipping was performed within 36 hours of presentation on patients who were clinically stable for surgery. Antihypertensive therapy was used to maintain a systolic blood pressure under 120 mmHg preoperatively. Nimodipine, 60 mg orally every 4 hours, was begun on admission and continued as recommended postoperatively.[6]

Results

Age, Past Medical History, Presenting Clinical Grade

The average age at presentation was 49 to 52 years with the exception of Caucasian females who had an average age of 62 years. The average weight in all four populations was 76 kg irrespective of sex. Relevant aspects of past medical history included diabetes, hypertension, chronic obstructive pulmonary disease, morbid obesity, and congestive heart failure. A comorbid health problem was present by history in 85% of African American females, 74% of African American males, 50% of Caucasian females, and 76% of Caucasian males. Significantly fewer Caucasian females had a comorbid health problem (χ-square = 4.3, p = .04). Clinical presentation was evaluated with the Hunt and Hess grading scale and modified for comorbid factors.[7] Average modified scores were 3.7 for African American females, 3.8 for African American males, 3.5 for Caucasian females, and 2.9 for Caucasian males.

Aneurysm Location and Multiplicity

The most common aneurysm location (59%) was on the anterior communicating artery.[8] Approximately 30% of aneurysms were located on the posterior communicating artery and 15% were located on the proximal middle cerebral artery. Multiple aneurysms were present in 16% of patients overall. Eighty-seven percent of these patients were female. There was no correlation between race and aneurysm location. The incidence of concurrent prehemorrhage health problems or the presenting neurological grade did not differ between patients with single or with multiple aneurysms. Thirty-five percent of the multiple aneurysms were located on one side of the circle of Willis, whereas 30% were bilateral. Multiple aneurysms were associated with a mortality rate of 46%.

Mortality

Many of the patients died from immediate complications of hemorrhage prior to operative intervention (15%). Preoperative mortality rates were as follows: 7% for African American females, 32% for African American males, 6% for Caucasian females, and 18% for Caucasian males. The difference between African American and Caucasian males was not statistically significant (χ-square = 0.93, p = .34). The difference between African American males and the combined group consisting of both African American and Caucasian females was statistically significant (χ-square = 6.0, p = .005). The 30-day postoperative mortality rates were 16% for African American females, 23% for African American males, 24% for Caucasian females, and 7% for Caucasian males. The differences among the 30-day postoperative mortality rates were not statistically significant (overall χ-square = 1.9, p = .60).

Hunt and Hess Grade

Patients who died preoperatively, 3.2 ± 0.3 for patients who died within 30 days postoperatively and 2.4 ±0.1 for patients who survived 30 or more days. The pairwise differences between these means are all statistically significant as assessed by Duncan's test with the comparisonwise error rate controlled at the

0.05 level (overall F = 19.5, p = .0001). For each unit increase in Hunt and Hess score, it is estimated that a patient has a 4.3 times (95% confidence interval = 2.1–9.0) greater chance of dying preoperatively. Given that a patient is operated upon, it is estimated that for each unit increase in Hunt and Hess grade, the patient has a chance 2.1 times (95% confidence interval = 1.2–3.8) greater chance of postoperative death within 30 days. Race and sex did not make significant additional contributions to risk of death above that predicted by Hunt and Hess grade.

Vasospasm

The postoperative development of mildly to moderately elevated transcranial Doppler flow velocities in the anterior circulation was 83% in the African American female population, 66% for the African American male population, 73% for Caucasian females, and 53% for Caucasian males. Elevated velocities on transcranial Doppler have been correlated with early vasospasm and were divided into mild (120–200 cm/sec) and severe (> 200 cm/sec).[9] Clinical vasospasm was determined by postoperative deterioration in neurological examination. The onset of clinical vasospasm correlated with the prior or concurrent development of elevated velocities by transcranial Doppler in all cases.

The survival rate in patients who developed vasospasm by transcranial Doppler was between 70 and 85% and did not differ statistically significantly between the patient groups. In patients who demonstrated clinical signs of vasospasm postoperatively, African American males had the lowest survival rate at 44%. African American females and Caucasian males shared similar survival rates of 75% and 80%, respectively. Caucasian females had a survival rate of 64%.

Electrolytes

Serum levels of sodium, potassium, and calcium were measured and recorded at the first sign of velocity elevation on transcranial Doppler and at the first sign of clinical vasospasm. The average sodium concentration for the group was 140 mEq/L, and the average ranged from 139 to 141 for the four patient groups. Potassium averages ranged from 3.7 to 4.1 mEq/L with an overall mean of 3.8. Mean serum calcium ranged from 8.4 to 9.6 mg/dL, with an average of 8.6. Concentrations of these electrolytes in those patients that subsequently died from vasospasm did not differ from the group as a whole. There were no statistical differences in the level of these electrolytes in patients who developed vasospasm based on clinical or Doppler criteria and patients who did not develop any sign of vasospasm.

Hydrocephalus

Eight of 96 patients required placement of an external ventricular drain during their hospitalization. All eight were placed on the day of presentation as treatment for acute hydrocephalus. Three of these patients died during hospitalization, the remaining five required subsequent permanent placement of a ventriculoperitoneal shunt.

Discharge Destination and Level of Function

General functional exams were performed for all four patient groups on discharge. The patients were stratified according to the ability to ambulate alone and unassisted or with the aid of a walker or cane, the necessity for a percutaneous gastric tube, and basic language function. Language function was defined by the ability to follow commands and communicate a reply to a verbalized question. Possible discharge destinations were home, nursing home, rehabilitation center, and the morgue. Fifty-two percent of patients were discharged to home, 15% went to a rehabilitation center prior to home discharge, three patients were placed in a nursing home on discharge, and 29% of patients died.

Discussion

Sex, Race, and SAH

Analysis of the patients who did not survive the hemorrhage showed a higher mortality for African American males in the preoperative period and for males in general overall. The population who perished was 47% male and 53% female, which does not correlate with the overall study population, which was 37% male and 63% female. The racial distribution was nearly the same for the entire population and for those who died.

Sex, Race, and Vasospasm

Two methods were used to record and grade the presence of vasospasm: elevated transcranial Doppler arterial flow velocities and deterioration of clinical status. The incidence of elevated transcranial Doppler flow velocities and clinical deterioration from vasospasm differed among the four groups. Postoperative vasospasm, based on Doppler criteria, was highest in the African American female population (81%) and lowest in Caucasian males (38%).

This study found that patients that developed clinical vasospasm who also had transcranial Doppler measurements usually demonstrated elevated flow velocities on transcranial Doppler. The correlation of vasospasm diagnosed by transcranial Doppler and

eventual or concurrent development of clinical vasospasm was lowest among African American women at 69%. The incidence ranged between 67% and 80% for the other three groups (no statistically significant differences). The mortality rate for Caucasian males who developed clinical vasospasm postoperatively was lowest at 20%. Corresponding rates were 36% for Caucasian females, 25% for African American females, and 56% for African American males. Overall, there was a 33% mortality rate for patients who developed clinical vasospasm.

Sex, Race, and Comorbid Factors

The presence of comorbid health problems; for example, hypertension, diabetes, or chronic obstructive pulmonary disease, has been associated with a poorer outcome after aneurysmal SAH. The high incidence of preexisting hypertension may be loosely correlated with patient sex and race. Overall, 64% of our patient population had a history of hypertension and 75% had a documented history of comorbid health problems. This study differs from previous studies in this respect where prehemorrhage hypertension rates are on average 43%.[10] African American females and males had 76% and 63% incidences, respectively, of preexisting hypertension and Caucasian females and males had preexisting incidences of 44% and 58%, respectively. Eighty-five percent of African American females and 74% of African American males possessed one or more documented comorbid factors prior to SAH.

Conclusion

Between the autumn of 1993 and the spring of 1999,96 patients presented to the University of Mississippi Medical Center emergency room with CT-diagnosed, arteriogram-verified anterior circulation aneurysmal SAH. Fifteen percent of these patients died of immediate complications from the hemorrhage prior to surgical intervention. Clinical signs of vasospasm were present in 30 to 70% of patients. Although reported in other studies, a relative hyponatremic state at the onset of vasospasm, diagnosed based on transcranial Doppler or clinical criteria, was not observed in any of the patient groups. We found the Hunt and Hess grading scale as well as the modified Hunt and Hess scale to correlate statistically with general patient outcome. The population as a whole had a high prevalence of prehemorrhage chronic medical illness, with 75% of patients having a known medical history of either or both hypertension and diabetes. Although the African American male mortality did not differ statistically significantly from the Caucasian male mortality, it was statistically higher than the combined female groups.

Many factors have been correlated with the mortality and morbidity of aneurysmal SAH. This study is limited by the small number of patients included. However, with the continued development of a comprehensive database, more definite conclusions may be drawn from these data. A continued prospective study is in progress to further evaluate this particular patient population.

Acknowledgment

This work was supported by grants from the American Heart Association Bugher Foundation and the National Institutes of Health.

REFERENCES

1. Broderick JP, Brott T, Tomsick T, Huster G, Miller R. The risk of subarachnoid and intracerebral hemorrhages in blacks as compared with whites. N Engl J Med 1992;326:733–736
2. Dennis GC, Welch B, Cole AN, et al. Subarachnoid hemorrhage in the African-American population: a cooperative study. J Natl Med Assoc 1997;89:101–108
3. Keller AZ. Hypertension, age and residence in the survival with subarachnoid hemorrhage. Am J Epidemiol 1980;91:139–147
4. Sacco RL, Wolf PA, Bharucha NE, et al. Subarachnoid and intracerebral hemorrhage: natural history, prognosis, and precursive factors in the Framingham Study. Neurology 1984;34: 847–854
5. Selman W, Taylor C, Tan R, et al. The neurosurgeon and the acute stroke patient in the emergency department: diagnosis and management. Clin Neurosurg 1999;45:74–85
6. Barker FG, Ogilvy CS. Efficacy of prophylactic nimodipine for delayed ischemic deficit after subarachnoid hemorrhage: a metaanalysis. J Neurosurg 1996;84:405–414
7. Hunt WE, Hess RM. Surgical risk as related to time of intervention in the repair of intracranial aneurysms. J Neurosurg 1968;28:14–20
8. Ohaegbulam SC, Dujovny M, Ausman JI, Diaz FG, Malik GM. Ethnic distribution of intracranial aneurysms. Acta Neurochir (Wien) 1990;106:132–135
9. Newell DW, Winn HR. Transcranial Doppler in cerebral vasospasm. Neurosurg Clin N Am 1990;1:319–328
10. Taylor CL, Yuan Z, Selman WR, Ratcheson RA, Rimm AA. Cerebral arterial aneurysm formation and rupture in 20,767 elderly patients: hypertension and other risk factors. J Neurosurg 1995;83:812–819

SECTION VIII

Clinical—Medical Management

54

Clinical Trial Dilemmas in Vasospasm

MARIO ZUCCARELLO, M.D. AND NORBERTO ANDALUZ, M.D.

Cerebral vasospasm after subarachnoid hemorrhage (SAH) is the leading cause of morbidity and mortality after the ruptured aneurysm has been successfully clipped or coiled. Vasospasm adversely affects 10 to 20% of patients with aneurysmal SAH.[1] Although vasospasm is present on catheter angiograms in 40 to 70% of patients with SAH, only 20 to 30% of patients will develop clinical vasospasm.[1] In most recent series, up to 15% of patients surviving the ictus of SAH experience stroke or death from vasospasm despite maximal medical therapy, including hemodynamic therapy and nimodipine. Manipulation of blood pressure, blood volume, and hematocrit or hypertensive, hypervolemic therapy has been called triple-H therapy but will be termed hemodynamic therapy in this chapter.[2]

Current strategies for the treatment of cerebral vasospasm aim at the reduction of the consequences of prolonged vasoconstriction from the point of view of antagonizing the vasoconstriction and of neuroprotection. Pharmacological agents effective in the prevention of vasospasm, however, remain elusive. A large number of clinical trials have been conducted to test the efficacy of drugs in the treatment and prevention of vasospasm, and many of these showed interesting results in subsets of patients. However, only nimodipine has been associated with relative reductions of 40 to 86% in poor outcome in patients experiencing SAH.[1,3] Despite the institution of these valuable therapies, there is a subset of patients who do not respond to these measures. This manuscript reviews the results of some of these trials and evaluates the reasons for their low success rate. The most important clinical trials have tested hemodynamic therapy, calcium channel antagonists, antioxidant and free radical scavengers, and intracisternal fibrinolytic agents. Some effort is made to grade these studies based on the commonly used method of grading of evidence from clinical trials.[4]

Hemodynamic Therapy

The combination of induced hypervolemia, hemodilution, and hypertension (often called triple-H therapy)[2] is based upon the assumption that, in the presence of cerebral vasospasm, cerebral blood flow pressure autoregulation is impaired and blood flow becomes pressure-dependent. The use of hemodynamic therapy after early surgery has been reported to improve outcomes, achieving reversal of symptoms of vasospasm in up to 60% of cases.[5] However, the reported studies involve only small numbers of patients and no adequate, randomized, blinded, controlled studies are available. Although it is difficult to determine the beneficial effect of hemodynamic therapy from such studies, evidence from small series suggests that this approach may decrease morbidity and mortality (level of evidence III-V, grade C recommendation). Common clinical practice consists of avoiding fluid restriction and supplying volume replacement to induce moderate hypervolemia and hypertension whereas hemodilution is not an integral part of the therapy in most centers.

Calcium Antagonists

Nimodipine

Nimodipine has been widely used as a prophylactic agent against delayed ischemic neurological deficits following SAH. This is based on results of several randomized trials.[3,6] A meta-analysis of all published randomized trials of prophylactic nimodipine included 1202 SAH patients.[6] Nimodipine use was associated with an improvement in the odds of good and of good plus fair outcomes by ratios of 1.9:1 and 1.7:1,

respectively ($p < .005$ for both measures). The odds of either or both deficit and mortality attributed to vasospasm and of cerebral infarction on cranial computed tomography were reduced by ratios of 0.5:1 to 0.6:1 in the nimodipine group ($p < .008$ for all measures). The combined rate of death and disability from vasospasm was reduced from 33% in the placebo group to 20% in the nimodipine group. Overall mortality was slightly reduced in the nimodipine group, but the trend was not statistically significant. Although the majority of individual trials examined did not show statistically significant differences between patients treated with nimodipine and those without at the $p < .01$ level, the meta-analysis suggested significant efficacy of prophylactic nimodipine in improving outcome after SAH under the conditions used in these trials. Most of the favorable effect of nimodipine was due to one study in which modern management of vasospasm probably was not employed and relatively few patients had aneurysms and underwent surgery, leaving open to question whether nimodipine would exert similar effects should it be tested in a randomized trial today.[3]

Nicardipine

From October 1987 to September 1989, 41 North American neurosurgical centers in the Cooperative Aneurysm Study accrued 906 SAH patients into a prospective, randomized, double-blind, placebo-controlled trial of high-dose intravenous nicardipine to test whether treatment with this agent improved overall outcome. Eligible patients received 0.15 mg/kg/hr of nicardipine or placebo by continuous infusion for up to 14 days following SAH. The incidence of symptomatic vasospasm during the treatment period was higher in the placebo group (46%) than in the nicardipine group (32%, $p < .001$). More patients in the placebo group had hemodynamic therapy for symptomatic vasospasm (38% of the placebo group vs 25% of the nicardipine group; $p < .001$) and antihypertensive agents were used less frequently in the nicardipine patients (26% of the nicardipine group vs 43% of the placebo group, $p < .001$). Despite the reduction in symptomatic vasospasm in the nicardipine group, overall outcome at 3 months was similar between the two groups. The reported rates of vasospasm diagnosed by transcranial Doppler ultrasound were 49% and 33% for the placebo and nicardipine patients, respectively ($p < .01$). Rates for angiographic vasospasm were 51% and 33% for placebo and nicardipine patients, respectively ($p < .01$). The combined death and disability from vasospasm was reduced from 12% in the placebo group to 8% in the nicardipine group.[7,8] Fifty-five percent of nicardipine patients were rated as having a good recovery according to the Glasgow outcome scale and 17% were dead at follow-up, compared with 56% and 18%, respectively, in the placebo group (not statistically significant). These data suggested that high-dose intravenous nicardipine treatment was associated with a reduced incidence of symptomatic vasospasm in patients with recent aneurysmal SAH, but not with an improvement in overall outcome at 3 months when compared with standard management.

A review of these studies suggests that high-dose intravenous nicardipine reduced the incidence of angiographic and symptomatic vasospasm in patients with aneurysmal SAH but that treatment was complicated by side effects such as hypotension, pulmonary edema, and azotemia. Therefore, another randomized, double-blind trial comparing high-dose (0.15 mg/kg/hr) nicardipine with a 50% lower dose (0.075 mg/kg/hr) administered by continuous intravenous infusion for up to 14 days following SAH was conducted. Patients in the high-dose group received a significantly smaller proportion of the planned dose than those in the low-dose group (80 ± 0.2% vs 86 ± 0.2%, $p < .05$), largely because of premature treatment termination after adverse medical events. The incidence of symptomatic vasospasm was 31% in both groups, and the overall 3-month outcomes were nearly identical. These data suggested that, from a clinical standpoint, the results of high-dose and low-dose nicardipine treatment are virtually equivalent, but administration of low-dose nicardipine is attended by fewer side effects.[9]

Fasudil

Fasudil, a potent vasodilator that probably exerts its effects by acting as a kinase inhibitor, particularly of Rho kinase, was tested in four studies in Japan. In the first study, reported in 1992, 267 patients in 60 centers received either 30 mg AT877 (fasudil) or a placebo (saline) by intravenous injection over 30 minutes, three times a day for 14 days following surgery. It was found that AT877 reduced angiographically demonstrable vasospasm by 38% (from 61% in the placebo to 38% in the fasudil group, $p = .002$), low-density regions on computed tomography associated with vasospasm by 58% (from 38 to 16%, $p = .001$), and symptomatic vasospasm by 30% (from 50 to 35%, $p = .025$). Furthermore, fasudil reduced the number of patients with a poor clinical outcome associated with vasospasm by 54% (from 26 to 12%, $p = .015$). There were no serious adverse events reported in the AT877 group.[10] A more recent study by the same authors using the same protocol in 287 SAH patients revealed that fasudil significantly decreased symptomatic vasospasm from 50 to 35% and angiographic spasm from 62% to 38%.[11] Cerebral infarction related to vasospasm decreased from 38 to 16%. The combined death and disability

from vasospasm was reduced from 26% in the placebo group to 12% in the fasudil group.

Antioxidant/Free Radical Scavengers

Tirilazad

Tirilazad mesylate, a synthetic nonglucocorticoid 21-aminosteroid was the first member of a new class of cytoprotective lipid peroxidation–inhibiting compounds to be selected for study in humans. Tirilazad was investigated in several animal models and shown to prevent SAH-induced cerebral vasospasm. In a Phase II trial, tirilazad was shown to be safe and to decrease delayed ischemic deficits.[12,13] A study conducted in Europe, Australia, New Zealand, and South Africa reported that tirilazad administered at a dose of 6 mg/kg/day was associated with better neurological outcome and lower mortality than that found with control patients.[12,13] Sex differences and drug interactions with anticonvulsant therapy were observed. Dilantin may have increased drug clearance and women may have more rapid metabolism of the drug. A trial of tirilazad conducted in North American centers did not confirm the beneficial effect of tirilazad despite the decrease in severity of symptomatic vasospasm by administration of the study drug (11% placebo, 6% tirilazad, $p = .008$) and in combined death and disability from vasospasm (13% in the placebo group, 8% in the tirilazad group, $p < .04$).

Ebselen

Ebselen is a seleno-organic compound with antioxidant activity that is believed to act through a glutathione peroxidase-like action. Ebselen was evaluated in a multicenter, placebo-controlled, double-blind clinical trial in 286 patients with SAH. The incidence of clinically diagnosed delayed ischemic neurological deficits was unaltered. A significantly better outcome, however, was observed after ebselen treatment compared with placebo ($p = .005$). There also was a decrease in the incidence and extent of low-density areas associated with ebselen use ($p = .032$). In conclusion, ebselen reduced brain damage in patients with delayed ischemic neurological deficits, although not the incidence of the delayed ischemia itself.[14]

Intracisternal Fibrinolytic Agents

Cisternal fibrinolytic clot therapy achieved by injection of recombinant tissue plasminogen activator into the subarachnoid space has been shown in a human study to decrease the incidence of angiographic and symptomatic vasospasm but not to affect outcome at 3 months (level of evidence III–V, grade B).[15] The performance of meticulous surgical toilette of the basal cisterns and insertion of a continuous cisternal lavage system with fibrinolytic agents (chiefly urokinase) has been reported by several Japanese authors who have substantially reduced their rates of symptomatic and angiographic vasospasm to figures as low as 3%.[16] The proposed mechanism is a reduction in the concentration of blood-derived spasmogenic agents in the cerebrospinal fluid in contact with the basal brain arteries attained by promoting early subarachnoid clot clearance. These techniques have been reported to result in clearance of blood volumes of up to 100 mL of blood and 1 g/day of hemoglobin. Furthermore, a drainage volume–dependent effect has been demonstrated. A drawback to this approach is, in addition to the significant logistic effort, the very high incidence of ventriculoperitoneal shunting (up to 48%) and an infection rate of up to 9%.

Other Proposed Treatments

Other treatments that have been studied in smaller numbers of patients and generally in an uncontrolled fashion include $MgSO_4$,[17] intraventricular sodium nitroprusside,[18] and controlled-release intracisternal papaverine[19] or nicardipine.[20] There is an ongoing trial of prophylactic transluminal balloon angioplasty for prevention of vasospasm.[21]

Conclusion

A review of the above clinical trials suggests that cerebral vasospasm is present angiographically in more than 50% of patients with aneurysmal SAH. Clinically evident or symptomatic vasosapsm continues to develop in more than 30% of cases. The combined death and disability rate directly related to vasospasm is still > 10%. Despite a level of evidence of only III-V, hemodynamic therapy is used commonly and may decrease morbidity and mortality from vasospasm. Clinical practice varies among countries and the use of hemodynamic therapy is not uniformly adopted worldwide. This may have influenced the results of previous clinical trials. Gender effects and drug interactions, for example with dilantin, may be very important factors as well, as suggested by the results of the tirilazad trials. These effects need to be taken into consideration in future studies. Preclinical studies must also be considered with caution.

Given the very low morbidity and mortality due to vasosapsm in the placebo groups of the nicardipine and tirilazad clinical trials, aneurysmal SAH treatment may have reached a zenith. It may be very difficult to demonstrate the protective effect of a novel pharmacological agent without randomizing large numbers of

patients. There may be a reluctance to do this on the part of industry and government because of the failure of neuroprotective agents in ischemic stroke trials. The difference in results of preclinical studies from those obtained in clinical practice have not engendered confidence that any benefits could therefore be achieved after potentially enormous financial commitments. More realistic end-point selection and new outcome measures such as psychological tests may need to be considered.

Finally, because current therapy treats only the consequences of vasospasm and we are as yet unable to prevent the vasoconstriction that is a primary process underlying vasospasm, basic research on molecular mechanisms of vasoconstriction and vasorelaxation must be pursued further. A highly focused, coordinated, multicenter approach should be developed to allow us to find a "silver bullet" to prevent or reverse vasospasm.

REFERENCES

1. Mayberg MR. Cerebral vasospasm. Neurosurg Clin N Am 1998;9: 615–627
2. Origitano TC, Wascher TM, Reichman OH, Anderson DE. Sustained increased cerebral blood flow with prophylactic hypertensive hypervolemic hemodilution ("triple-H" therapy) after subarachnoid hemorrhage. Neurosurgery 1990;27:729–739
3. Pickard JD, Murray GD, Illingworth R, et al. Effect of oral nimodipine on cerebral infarction and outcome after subarachnoid hemorrhage: British aneurysm nimodipine trial. BMJ 1989;298:636–642
4. Cook DJ, Guyatt GH, Laupacis A, et al. Rules of evidence and clinical recommendations on the use of antithrombotic agents. Chest 1992;102:305S–311S
5. Awad IA, Carter LP, Spetzler RF, Medina M, Williams FC Jr. Clinical vasospasm after subarachnoid hemorrhage: Response to hypervolemic hemodilution and arterial hypertension. Stroke 1987;18:365–372
6. Barker FG, Ogilvy CS. Efficacy of prophylactic nimodipine for delayed ischemic deficit after subarachnoid hemorrhage: a metaanalysis. J Neurosurg 1996;84:405–414
7. Haley EC Jr, Kassell NF, Torner JC. A randomized controlled trial of high-dose intravenous nicardipine in aneurysmal subarachnoid hemorrhage: a report on the cooperative aneurysm study. J Neurosurg 1993;78:537–547
8. Haley EC Jr, Kassell NF, Torner JC. A randomized trial of nicardipine in subarachnoid hemorrhage: angiographic and transcranial Doppler ultrasound results: a report of the Cooperative Aneurysm Study. J Neurosurg 1993;78:548–553
9. Haley EC Jr, Kassell NF, Torner JC, Truskowski LL, Germanson TP. A randomized trial of two doses of nicardipine in aneurysmal subarachnoid hemorrhage: a report of the Cooperative Aneurysm Study. J Neurosurg 1994;80:788–796
10. Shibuya M, Suzuki Y, Sugita K, et al. Effect of AT877 on cerebral vasospasm after aneurysmal subarachnoid hemorrhage: results of a prospective placebo-controlled double-blind trial. J Neurosurg 1992;76:571–577
11. Shibuya M, Asano T, Sasaki Y. Effect of fasudil HC1, a protein kinase inhibitor, on cerebral vasospasm. Acta Neurochir Suppl 2001;77:201–204
12. Lanzino G, Kassell NF, Dorsch NW, et al. Double blind, randomized, vehicle-controlled study of high-dose tirilazad mesylate in women with aneurysmal subarachnoid hemorrhage, I: A cooperative study in Europe, Australia, New Zealand, and South Africa. J Neurosurg 1999;90:1011–1017
13. Lanzino G, Kassell NF. Double blind, randomized, vehicle-controlled study of high-dose tirilazad mesylate in women with aneurysmal subarachnoid hemorrhage, II: A cooperative study in North America. J Neurosurg 1999;90:1018–1024
14. Saito I, Asano T, Sano K, et al. Neuroprotective effect of an antioxidant, ebselen, in patients with delayed neurological deficits after aneurysmal subarachnoid hemorrhage. Neurosurgery 1998;42:269–277
15. Findlay JM, Kassell NF, Weir BK, et al. A randomized trial of intraoperative, intracisternal tissue plasminogen activator for the prevention of vasospasm. Neurosurgery 1995;37:168–176
16. Kodama N, Matsumoto M, Sasaki T, Konno Y, Sato T. Cisternal irrigation therapy with urokinase and ascorbic acid for prevention of vasospasm. Acta Neurochir Suppl 2001;77:171–174
17. Veyna RS, Seyfried D, Burke DG, et al. Magnesium sulfate therapy after aneurysmal subarachnoid hemorrhage. J Neurosurg 2002;96:510–514
18. Thomas JE, McGinnis G. Safety of intraventricular sodium nitroprusside and thiosulfate for the treatment of cerebral vasospasm in the intensive care unit setting. Stroke 2002;33:486–492
19. Dalbasti T, Karabiyikoglu M, Ozdamar N, Oktar N, Cagli S. Efficacy of controlled-release papaverine pellets in preventing symptomatic cerebral vasospasm. J Neurosurg 2001;95:44–50
20. Kasuya H, Onda H, Takeshita M, Okada Y, Hori T. Efficacy and safety of nicardipine prolonged-release implants for preventing vasospasm in humans. Stroke 2002;33:1011–1015
21. Muizelaar JP, Zwienenberg M, Rudisill NA, Hecht ST. The prophylactic use of transluminal balloon angioplasty in patients with Fisher grade 3 subarachnoid hemorrhage: a pilot study J Neurosurg 1999;91:51–58

55

Circulating Blood Volume After Subarachnoid Hemorrhage

HIDETOSHI KASUYA, M.D., HIDEAKI ONDA, M.D., ATSUSHI SASAHARA, M.D., HIROYUKI AKAGAWA, M.D., TAKU YONEYAMA, M.D., TOMOKATSU HORI, M.D.

Abstract

Maintenance of an adequate intravascular volume is important in the management of patients with subarachnoid hemorrhage (SAH). This study investigated the circulating blood volume (CBV) following SAH using indocyanine green pulse spectrophotometry. Circulating blood volume and plasma hormones related to stress and fluid regulation were measured four times (day 2–3, 4–5, 7–8, and 14) in 50 consecutive patients with SAH who were treated surgically within 48 hours. The control CBV was 72 mL/kg. The mean value of CBV was 64 mL/kg on day 2 to 3. This value gradually increased to 69 mL/kg on day 4 to 5,71 mL/kg on day 7 to 8, and 70 mL/kg on day 14 ($p = .005$). The clinical grades and plasma adrenocorticotropic hormone levels were higher in patients with CBV <60 mL/kg on day 2 to 3 compared with those with higher values ($p < .05$). There were no significant differences in other physiological and laboratory parameters such as time to surgery, estimated blood loss, levels of plasma noradrenaline, brain natriuretic peptide, serum sodium, and hematocrit. When CBV decreased by more than 10% of the level present at an earlier time, there were decreases in hematocrit ($p < .05$), serum sodium ($p < .01$), and serum albumin ($p < .05$) and an increase in urine sodium ($p < .05$). A significant reduction of CBV was noted following SAH and early surgery especially in poor grade patients. This decrease in CBV could not be detected by routine examinations. Anemia, central salt wasting, and hypoalbuminemia may be related to a decrease in CBV occurring days after SAH. Indocyanine green pulse spectrophotometry could be a powerful tool for the management of patients with SAH.

Maintenance of an adequate intravascular volume is important in the management of patients with aneurysmal subarachnoid hemorrhage (SAH). However, no monitoring system has been used for estimating circulating blood volume (CBV) other than the Swan-Ganz catheter and radioactive isotopes. Indocyanine green pulse spectrophotometry has been developed to facilitate minimally invasive measurement of CBV by progressively estimating the arterial concentration of indocyanine green. We reported that indocyanine green pulse spectrophotometry could be used to demonstrate that the CBV decreased postoperatively to approximately four fifths of its preoperative value and gradually increased and returned to its preoperative value on day 7 following craniotomy in patients with aneurysmal SAH.[1]

This study investigated changes in CBV after SAH and early surgery using indocyanine green pulse spectrophotometry. The mechanism of the change in CBV following SAH was examined by measuring hormones related to stress and fluid regulation.

Patients and Methods

Fifty consecutive patients (14 men and 36 women, mean age ± standard deviation of 60 ± 13 years) with SAH were investigated in the Department of Neurosurgery at Tokyo Women's Medical University. Patients were eligibile if they underwent surgery for clipping of a ruptured cerebral aneurysm within 48 hours of SAH. Patients were accrued from among 77 SAH patients hospitalized between October 1, 1999, and December 2002. Basic management of postoperative patients followed previously described procedures.[2]

All patients underwent measurements of CBV, body weight, plasma noradrenaline, adrenocorticotropic hormone (ACTH), Cortisol, aldosterone, atrial natriuretic peptide, and brain natriuretic peptide and red blood cell mass, hemoglobin, and hematocrit. Albumin, serum sodium and potassium, and urine sodium were also measured. Each of these factors was measured at least four times and values were grouped according to whether they were obtained 2 to 3,4 to 5, 7 to 8, or 14 days after the onset of SAH.

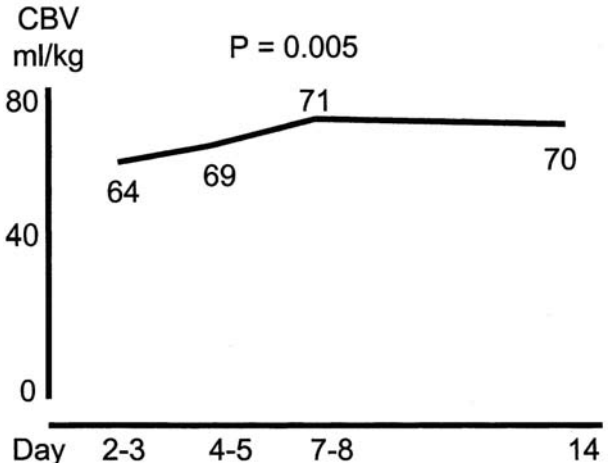


FIGURE 55–1 Serial change in the circulating blood volume (CBV) between 2 to 3, 4 to 5, 7 to 8, and 14 days after aneurysmal subarachnoid hemorrhage and surgery. There was a significant change over time in CBV ($p = .005$).

Results

The mean value of CBV 2 to 3 days after SAH was 64 mL/kg. This value increased to 69 mL/kg on day 4 to 5, 71 mL/kg on day 7 to 8, and 70 mL/kg on day 14 (Fig. 55–1). There was a significant change over time ($p = .005$). In the early postoperative stage (day 2–3), CBV generally ranged between 32 mL/kg and 97 mL/kg. The World Federation of Neurological

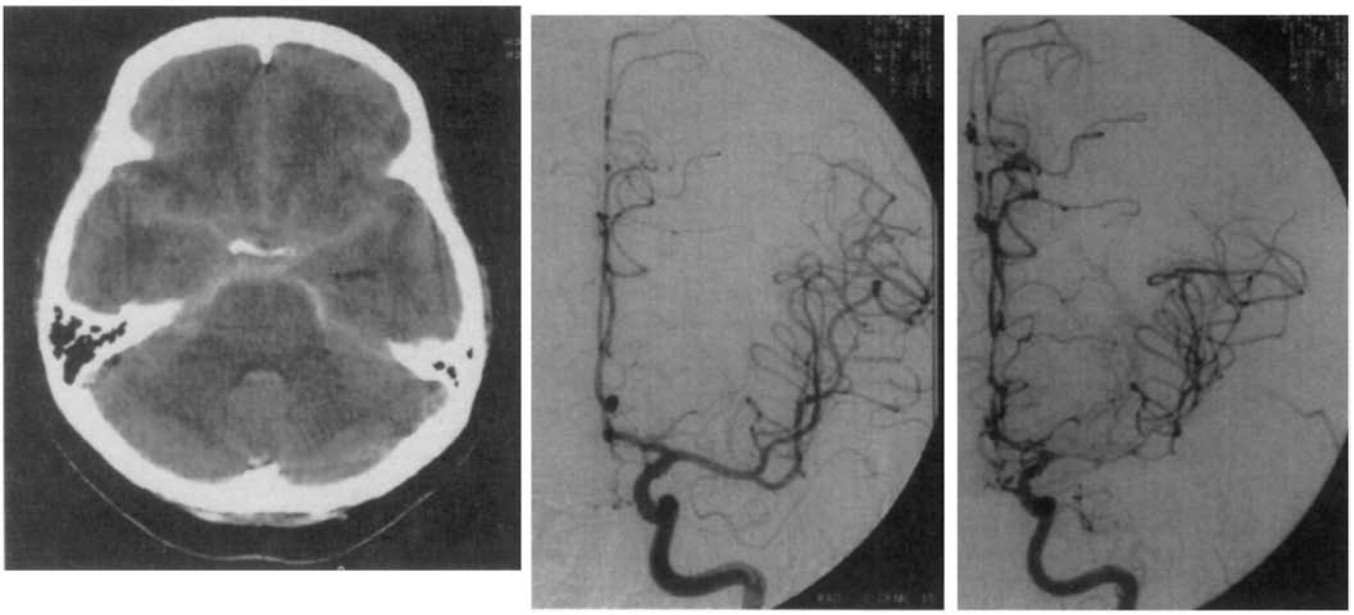


FIGURE 55–2 Preoperative computed tomographic scan of a 56-year-old female shows diffuse, thick clots in the basal cisterns (left). Initial cerebral angiography showed an aneurysm arising from the junction of the basilar and left superior cerebellar arteries. This aneurysm was clipped through a left frontotemporal craniotomy. Comparison of initial (middle) angiography of the left internal carotid artery with that obtained 10 days after SAH (right) shows severe angiographic vasospasm of the left proximal middle cerebral artery.

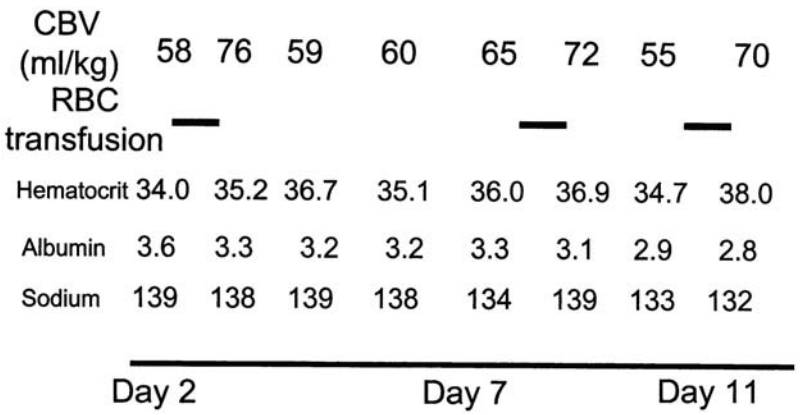


FIGURE 55–3 The time course of circulating blood volume (CBV), hematocrit, albumin, and serum sodium in the patient shown in Figure 55–2. Red blood cell transfusion increased CBV effectively.

Surgeons (WFNS) clinical grade,[3] time to surgery, estimated blood loss, and physiological and laboratory parameters were compared in patients with CBV less than and greater than 60 mL/kg. There were significant differences in the WFNS grade ($p < .05$) and ACTH levels ($p < .05$) between the two groups.

Laboratory and physiological parameters were compared in the patients who did and did not experience decreases of more than 10% in their CBV. The mean value of CBV of 71 mL/kg decreased to 57 mL/kg. There were significant changes in hematocrit ($p < .05$), serum sodium ($p < .01$), urine sodium ($p < .05$), and serum albumin ($p < .05$) between the two CBV values. Twenty-four patients received transfusions of red blood cells over the time that CBV dropped more than 10%. The average volume of erythrocyte transfusion was 3.2 ±1.0 unit (one unit erythrocytes equals erythrocytes from approximately 200 mL blood), and this transfusion resulted in a significant increase in mean CBV from 61 mL/kg to 70 mL/kg ($p < .0001$). The mean values for the hematocrit before and after blood transfusion were 34 and 35% (not significant, Figs. 55–2 and 55–3).

Discussion

A significant decrease in CBV was noted in the postoperative stage in patients with SAH treated by early surgery. The initial decrease in CBV might be related to the intensity of the initial stress in addition to the surgical stress. The initial decrease following SAH and early surgery is probably due to shift of fluid to the interstitial space.[4] No routine clinical or laboratory examinations were found to be useful for estimating the CBV during the postoperative stage (day 2–3), despite substantial variation in CBV between 32 and 97 mL/kg.

When CBV fell by more than 10% of the former level, there were decreases in hematocrit and serum albumin and sodium and an increase in urine sodium. These results suggest that depletion of red blood cells, cerebral salt wasting, and hypoalbuminemia are related to the cause of the decrease in CBV that occurs after the initial reduction. The reduction of CBV following SAH may not be a simple phenomenon that can be explained by a single hormonal change.

To achieve normovolemia during vasospasm in SAH patients, the depleted erythrocyte volume can be treated by red blood cell transfusion. Hypoalbuminemia can be remedied by adding human albumin and central or cerebral salt wasting by replacement of sodium chloride with fluid or by administration of fludrocortisone.[3] Our present practice is to measure CBV postoperatively using indocyanine green pulse spectrophotometry. We measure CBV repeatedly when the initial CBV is < 60 mL/kg. Circulating blood volume is also measured when transfusions of erythrocytes and albumin are considered, when hyponatremia shows progression, and when symptoms of vasospasm are encountered. We suggest that this management scheme can avoid unnecessary transfusions of erythrocytes and albumin.

REFERENCES

1. Kasuya H, Onda H, Yoneyama T, Sasaki T, Hori T. Bedside monitoring of circulating blood volume after subarachnoid hemorrhage. Stroke 2003;34:956–960
2. Hirasawa K, Kasuya H, Hori T. Change in circulating blood volume following craniotomy J Neurosurg 2000;93:581–585
3. Drake CG, Hunt WE, Sano K, et al. Report of World Federation of Neurological Surgeons Committee on a Universal Subarachnoid Hemorrhage Grading Scale. J Neurosurg 1988;68:985–986
4. Kasuya H, Kawashima A, Namiki K, Shimizu T, Takakura K. Metabolic profiles of patients with subarachnoid hemorrhage treated by early surgery. Neurosurgery 1998;42:1268–1275

56

Cerebrovascular Effects of Hypertonic Saline in Poor Grade Patients with Subarachnoid Hemorrhage

MING-YUAN TSENG, M.D., PIPPA AL-RAWI, B.SC, JOHN D. PICKARD, M.D., FRANK A. RASULO, M.D., PETER J. KIRKPATRICK, M.B., CH.B., F.R.C.S. (S.N.)

Abstract

This study tested the hypothesis that a bolus infusion of hypertonic saline improves cerebral perfusion in poor grade subarachnoid hemorrhage (SAH) patients without compromising flow to other areas. The effects of intravenous administration of 23.5% hypertonic saline (2 mL/kg) to 10 patients (World Federation of Neurological Surgeons grades 4 and 5) were monitored using transcranial Doppler, intracranial pressure, serum sodium and osmolality, and regional cerebral blood flow (CBF). Regional CBF was measured with xenon computed tomography (CT). Cerebral perfusion pressure increased 27%, arterial blood pressure increased 11%, intracranial pressure fell by 75%, and middle cerebral artery flow velocity increased by 71% after infusion of hypertonic saline. Xenon CT confirmed the increase in CBF (23% increase) and that this occurred without producing regional differences. Serum sodium rose by 11 mmol/L, osmolality increased by 27 mosm/L, and hemoglobin decreased by 0.7 g/L. In conclusion, a bolus of 23.5% hypertonic saline increases CBF in poor grade patients with SAH and also improved indices of blood rheology

Cerebral blood flow (CBF) is reduced globally following subarachnoid hemorrhage (SAH), particularly in poor grade patients.[1] Cerebral vasospasm, hydrocephalus, raised intracranial pressure, hypoxia, hyponatremia, hypotension, fever, and seizures may further compromise the cerebral circulation leading to infarction and poor outcome. In view of the beneficial effects of hypertonic saline on intracranial pressure and cerebral perfusion pressure in patients with brain injury,[2,3] we wished to define the effects on CBF in poor grade patients following SAH. A full report of the work has been published.[4]

Patients and Methods

The study was approved by the local Research Ethics Committee. Ten patients ranging in age from 19 to 80 years were admitted with a World Federation of Neurological Surgeons grade 4 to 5 SAH.[5] Patients were studied within the environment of the neurosurgical critical care unit. Exclusion criteria included traumatic SAH, an initial serum sodium > 155 mmol/L, initial serum osmolality > 320 mmol/L, and impaired renal or cardiovascular function.

Mean arterial blood pressure, heart rate, central venous pressure, hourly urine output, fluid balance, and tympanic temperature were routinely monitored together with intracranial pressure. Intracranial pressure was monitored via a microtransducer or external ventricular drain. Bilateral transcranial Doppler measurements were made of middle cerebral artery flow velocity.

CBF was measured with xenon computed tomography (CT) using 28% xenon gas. Immediately after the baseline scan, 2 mL/kg of 23.5% hypertonic saline was infused via a central venous catheter over 20 minutes. Ten minutes after completion of this infusion, a second xenon CT was acquired. When the patient had an oxygen requirement > 55%, infusion was monitored by transcranial Doppler only. The full details of the methods have been published.[4]

Results

Cerebral perfusion pressure increased by 27% (p = .0003 at 28 minutes), and this was associated with a rise in mean arterial blood pressure of 11% (p = .02 at 22 minutes) and a fall in intracranial pressure of 75% (p = .002 at 60 minutes). Middle cerebral artery flow velocity increased by 71% (p = .00005 at 20 minutes). Hence, the estimated cerebrovascular resistance fell by 27% (p = .01 at 16 minutes). The half-lives of the effects on arterial blood pressure, cerebral perfusion pressure, intracranial pressure, flow velocity, and estimated cerebrovascular resistance were 20, 54, 139, 43, and 27 minutes, respectively. Xenon CT confirmed that the increase in CBF (23%, p = 0.02) displayed no regional differences. Serum sodium rose by 11 mmol/L and osmodality by 27 mmol/L. Hemoglobin decreased by 0.7 g/L and hematocrit decreased by 2%.

Discussion

These results show that a bolus infusion of 23.5% hypertonic saline over a short time increased CBF as documented by both transcranial Doppler and xenon CT methods. Compounding factors including arterial carbon dioxide tension, arterial oxygen tension, and temperature all remained stable during the investigations. Although CBF increases were seen for the vast majority of regions examined, infarcted tissues with a regional CBF of < 18 mL/100 g/min showed no change in CBF or in some cases showed a decrease. There was one example where CBF significantly fell with hypertonic saline, so it is not possible to say that intracerebral steal never occurs with hypertonic saline. A larger study would be required to answer this question. There was no evidence of a rebound increase in intracranial pressure (ICP) after the ICP-lowering effect of this single dose of hypertonic saline. The effect of hypertonic saline appears to reflect the reduction in cerebrovascular resistance, an increase in cerebral perfusion pressure, and an improved hemorheology A limitation of the use of hypertonic saline is that repeated treatment is restricted by the degree of hypernatremia that can develop. Further work is required to define the cerebral metabolic effects of hypertonic saline. Such studies would likely use tissue oxygen electrodes, microdialysis, and triple oxygen positron emission tomography.

Acknowledgment

We thank Dr. H. K. Richards for statistical advice. Supported by a Medical Research Council Program Grant for the Study of Acute Brain Injury.

REFERENCES

1. Grubb RJ Jr, Raichle ME, Eichling JO, Gado MH. Effects of subarachnoid haemorrhage on cerebral blood volume, blood flow and oxygen utilization in humans. J Neurosurg 1977;46:446–453
2. Sarez JI, Qureshi AI, Bhardwaj A, et al. Treatment of refractory intracranial hypertension with 23.4% saline. Crit Care Med 1998;26:1118–1122
3. Shackford SR, Bourguigan PR, Wald SL, et al. Hypertonic saline resuscitation of patient with head injury: a prospective, randomised clinical trial. J Trauma 1998;44:50–58
4. Tseng M-Y, Al-Rawi PG, Pickard JD, Rasulo FA, Kirkpatrick PJ. Effect of hypertonic saline on cerebral blood flow in poor grade patient with subarachnoid hemorrhage. Stroke 2003;34:1389–1396
5. Drake CG, Hunt WE, Sano K, et al. Report of World Federation of Neurological Surgeons Committee on a Universal Subarachnoid Hemorrhage Grading Scale. J Neurosurg 1988;68:985–986

57

Panax *Notoginseng Saponins for Prevention of Vasospasm and Delayed Ischemic Neurological Deficits After SAH: A Review*

LIQUN ZHONG, M.D., SULUN SUN, M.D., LIYUN HE, PH.D., ZHUANG ZHANG, PH.D.

Abstract

Multiple factors contribute to delayed cerebral vasospasm after aneurysmal subarachnoid hemorrhage (SAH). Current therapy of cerebral vasospasm consists of treating the consequences of vasoconstriction, and there is no drug that prevents development of vasospasm. *Panax* notoginseng is an herb whose roots are used in Chinese medicine. *Panax* notoginseng saponins (PNS) are the active components of *Panax* notoginseng. PNS consists of nine saponins of which NS Rb1 and PNS Rg1 have been introduced to this clinic. We present a review of the literature on PNS suggesting a possibility of its clinical use for prevention of delayed vasospasm and as a treatment of delayed ischemic neurological deficits. The medical literature was searched to find all references to vasospasm after SAH and cerebral hemorrhage. Online searches of the Chinese biomedical literature database and of Medline were performed using the keywords *sanchi (PNS), vasospasm, subarachnoid,* and *hemorrhage.* We review five effects of PNS on delayed vasospasm and delayed ischemia. These include effects on cerebral hemorrhage and vasospasm, vascular smooth muscle cells, blood clot, iron (Fe), and neurons (neuroprotection). PNS decreases blood clot and reduces the concentration of Fe^{2+} thereby preventing vascular endothelial cell and smooth muscle cell damage. It also dilates microvessels by blocking Ca^{2+} channels leading to an increase in local blood flow. PNS improves local metabolism of neurons and glial cells. Each of the mechanisms may influence the time course or the degree of vasospasm as well as clinical outcome after SAH.

Multiple factors contribute to delayed cerebral vasospasm after aneurysmal subarachnoid hemorrhage (SAH).[1] A key factor is hemoglobin released from the blood clot in the vicinity of the circle of Willis.[2] Current therapy of cerebral vasospasm consists of treating the consequences of vasoconstriction, and there is no drug that prevents development of vasospasm.[3] For many years, traditional medicine has been used for cerebral vascular diseases in China. According to a meta-analysis, *Panax* notoginseng, *Rheum,* and leeches are the most commonly used methods to treat acute intracranial hemorrhage.[4] *Panax* notoginseng saponins (PNS) are the active components of *Panax* notoginseng. PNS consists of nine saponins (Rb_1, Rb_2, Rc, Rd, Re, Rf, Rg_1, Rg_2, Rh). Recently, Rb_1 and Rg_1 have been introduced to this clinic. The mechanism of their

action is unclear but it seems that PNS may protect the brain and produce thrombolysis and vasodilation. We present a review of the literature on PNS suggesting a possibility of its clinical use for prevention of delayed vasospasm and as a treatment for delayed ischemic neurological deficits.

Materials and Methods

The medical literature was searched to find all references to vasospasm after SAH and cerebral hemorrhage. Online searches of the Chinese biomedical literature database and Medline were performed using the keywords *sanchi (PNS), vasospasm, subarachnoid,* and *hemorrhage.* References were included if they described the relevant use of PNS.

Results

PNS, Cerebral Hemorrhage, and Vasospasm

Injection of PNS has been shown to improve neurological outcome in acute cerebral hemorrhage.[5] It also has been reported to accelerate absorption of intracerebral hematomas caused by hypertension.[6] Furthermore, PNS decreased permeability of the blood–brain barrier, limited cerebral edema, and improved cerebral blood flow in an ischemia-reperfusion model in rats.[7] *Panax* notogingseng decoction has been used to treat vasospasm in a rabbit model of SAH.[8,9] This treatment accelerated subarchnoid clot lysis and improved neurological function.

PNS and Vascular Smooth Muscle Cells

Wu and Chen reported that PNS has vasodilatory effects.[10] According to the authors, PNS acts as a calcium (Ca^{2+}) channel blocker because intracellular Ca^{2+} release is inhibited by Rg_1 and R_e and extracellular Ca^{2+} influx is blocked by Rb_1. Furthermore, PNS produced vasodilation and an increase in cerebral blood flow in rats and rabbits.[11] Guan and colleagues reported in 1994 that PNS selectively inhibits Ca^{2+} entry through receptor-operated Ca^{2+} channels without affecting intracellular Ca^{2+} release or Ca^{2+} entry through voltage-dependent Ca^{2+} channels.[12]

The effect of PNS (50–800 mg/L) on synaptosomal ^{45}Ca uptake has been measured in vitro.[13] PNS produced a dose-dependent inhibition of Ca^{2+} uptake [IC_{50} = 111 (46–176) mg/L]. Both initial and maximal uptakes were inhibited. Similar effects were observed after administration of PNS, 200 mg/kg, intraperitoneally. The blocking effect of PNS was reversed by adding Ca^{2+} to the media in experiments in vitro. PNS also lowered the intracellular free Ca^{2+} concentration in dissociated neurons in response to addition of KCl (50 mmol/L), glutamate (100 μmol/L), or hypoxia.[14] Levels of Ca^{2+} and calmodulin in the blood and brain were examined by radioimmunoassay and atomic absorption spectrometry in a craniocerebral injury model in rabbits.[15] PNS administration reduced the Ca^{2+} and calmodulin contents in the blood and the brain.

PNS and Blood Clot

The powder Radix Panax Notoginseng inhibits thrombosis produced by thrombin factor (rat cerebral thrombin activator) in the mouse cerebral circulation.[16] This effect is dose dependent. In addition, erythrocyte intravascular flow velocity is increased after administration of PNS to mice, which further improves the blood circulation and assists in preventing thrombosis.[17] PNS also inhibits aggregation of platelets via its active component Rg1, which increases the levels of cyclic adenosine monophosphate in platelets and decreases the concentration of thromboxane A_2.[18]

PNS and Brain Protection

PNS has been shown to decrease brain oxygen consumption during hypoxia in humans.[19] At intravenous doses of 25 to 50 mg/kg, it dilates microvessels and increases cerebral blood flow in mice in vivo.[20] Higher doses (50 and 100 mg/kg for 3 days given intraperitoneally) decreased the breakdown of adenosine triphosphate in ischemic tissue by 58 and 45%, respectively. Thus, PNS modulates local blood flow and protects energy stores after ischemia. Study in vitro of primary cultured rat cortical neurons has shown that PNS (25 to 50 mg/L) attenuates neuronal damage during hypoxia/reoxygenation.[21,22] This was shown by PNS-related decreases in lactate dehydrogenase release and reduction in pathological changes in the cells. Moreover, PNS protected neurons from the excitotoxic effect of glutamate.

In our laboratory, we used vascular endothelial cells (ECV304) to examine effects of PNS. Toxicity was tested by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay, trypan blue staining, and lactate dehydrogenase levels. We observed decreased cell death, less lactate dehydrogenase release, and a significant increase in cell survival in cells exposed to PNS compared with control cells ($p < .001$).[23] Also, PNS protected rat astroglial cells from MTT toxicity and promoted proliferation and activity of the cells in the dose-dependent manner (unpublished observations).

Discussion

There has been only one clinical study examining the effect of PNS on vasospasm after SAH. Li et al reported

that a complex herbal decoction consisting mainly of Radix Panax Notoginseng decreased vasospasm by increasing the absorption of subarachnoid hematoma, decreasing neurotoxicity of nitric oxide and improving blood flow to hypoxic brain tissue.[9] Radix Panax Notoginseng consists of several components but a major component (~12%) are the saponins.[24] Some of those saponins have been identified and purified and their molecular structure, pharmacodynamics, pharmacokinetics, and effects on the nervous, cardiovascular, and immune systems studied.[25] There are also studies of their effects on platelets, blood sugar, inflammation, and aging. The most important effect of PNS on the brain is protection against cerebral ischemia and hypoxia.

Development of delayed cerebral vasospasm after SAH depends on many factors. However, the presence of hemoglobin in the subarachnoid space is recognized as the main risk factor for vasospasm. Contemporary treatment of vasospasm is focused on improvement of cerebral blood flow by using hemodynamic therapy. This therapy, however, requires expensive intensive care and it has the potential for serious side effects such as a hemorrhagic brain infarction, pulmonary edema, and heart failure. Intracisternal administration of fibrinolytic drugs such as tissue plasminogen activator and urokinase is effective at dissolving subarachnoid clot and reducing vasospasm, especially in combination with cerebrospinal fluid drainage, but there may be an increased risk of hemorrhage. Neuroradiological interventions such as cerebral transluminal angioplasty are also used to treat vasospasm but are dependent on the ready availability of a neuroradiological specialist. This may be difficult to do in some hospitals such as ours. The main accepted treatment for vasospasm, which was introduced in the 1980s, is the Ca^{2+} antagonist, nimodipine. Nimodipine treatment was reported to reduce the number of patients affected by cerebral vasospasm to 5 to 7% of the aneurysmal SAH population.[3]

PNS has many potential antivasospastic effects including reducing subarachnoid blood clot, dilating micro vessels, blocking Ca^{2+} channels, increasing local blood flow, and improving local metabolism of neurons and glial cells. Each of the aforementioned mechanisms may influence the time course or the degree of vasospasm as well as the clinical outcome after SAH. Although the usefulness of PNS for treatment of vasospasm after SAH remains to be rigorously tested in a vasospasm model before being studied in clinical trials, the early results of our ongoing clinical trial with PNS administration to patients after ischemic stroke strongly support our hypothesis.

REFERENCES

1. Macdonald RL, Weir BK, Runzer TD, et al. Etiology of cerebral vasospasm in primates. J Neurosurg 1991;75:415–424
2. Mayberg MR, Okada T, Bark DH. The role of hemoglobin in arterial narrowing after subarachnoid hemorrhage. J Neurosurg 1990;72:634–640
3. Seiler RW, Binggeli R. Is cerebral vasospasm still a clinical problem? Acta Neurochir (Wien) 2001;77:1–4
4. Guo JW, Liu MJ. Meta-analysis of acute intracerebral hemorrhage treated with traditional Chinese medicine or/and composition of promoting blood circulation and removing blood stasis. J China-Japan Friendship Hosp 2001;15: 283–286
5. Han GS, Lei T, Xu XL. Maximum dose of PNS injection to treat 60 cases of acute cerebral hemorrhage. Shanxi Zhong Yi 2002;23: 113–114
6. Guo YJ, Liang H. The effect of PNS on hematoma absorption after hypertension caused cerebral hemorrhage. J Fujian Coll Trad Chinese Med 1997;7:8
7. Liu J, Ji F, Wang T, et al. The effects of notoginsengoside on blood brain barrier and cerebral blood flow during reperfusion of cerebral ischemia in rats. Hebei Med J 2002;24:249–250
8. Otsuji T, Endo S, Hirashima Y, et al. An experimental model of symptomatic vasospasm induced by oxyhemoglobin in rabbits. Stroke 1994;25:657–662
9. Li HQ, Li J, Li F, Xue DP. The pharmacodynamics of Qingtong Sanchi Decoction on vasospasm after SAH. J Emerg Trad Chinese Med 2001;10:98–101
10. Wu JX, Chen JX. Depressant actions of Panax notogingseng saponins on vascular smooth muscles. Zhongguo Yao Li Xue Bao 1988;9:147–152
11. Vu JX, Sun JJ. Comparative effects of *Panax* notoginseng saponis, verapamil and norepinephrine on cerebral circulation in anesthetized rats and rabbits. Acta Pharmacol Sinca 1992; 13:520–523
12. Guan YY, Kwan CY, He H, Sun JJ, Daniel EE. Effects of *Panax* notoginseng saponins on receptor-operated Ca^{2+} channels in vascular smooth muscle. Zhongguo Yao Li Xue Bao 1994; 15:392–398
13. Ma LY, Xiao PG. Effect of saponins of *Panax* notoginseng on synaptosomal ^{45}Ca uptake. Zhongguo Yao Li Xue Bao 1997; 18:213–215
14. Ma LY, Xiao PG. Effects of *Panax* notoginseng on intracellular free Ca^{2+} concentration in dissociated neurons. Chin Pharm J 1998;33:467–469
15. Han JHW, Sun Z. Effect of *Panax* notogingseng saponin on Ca^{2+} and calmodulin in craniocerebral injury. Chinese J Integrated Trad Western Med 1999;19:227–229
16. Du L, Guo Y, Ma L, Yu L, Sun S, Jin W. Hemostasis and hemiaction of Radix Notoginseng, III: Effect of notoginseng on thrombosis in mice systemic circulation by rat cerebral thrombin activator (RCTA). Pharmcol Clin Chinese Materia Medica 1995; 5:26–28
17. Du L, Ma L, Yu L, Sun S, Jin W. Hemostasis and hemiaction of Radix Panax Notoginseng, III: Effect of Radix Panax Notoginseng on the microcirculation of mice's meninges in vivo. Chinese Pharmacol Clin 1995;6:25–27
18. Shen Y. Pharmacology of Chinese Herbs. Shanghai, China: Shanghai Science and Technology Publishing House; 1997: 120–122
19. Chen QK, Li L. The influence of *Panax* notoginseng saponin on microdialysis of brain damage by acute hypoxia in clinic. Chinese J Pathophysiol 1990;6:472–476
20. Ma LY, Wang CL, Zhang Q, Du LJ, Chen JM, Xiao PG. Effects of PNS on blood supply and on energy metabolism in mice brain tissue. Chinese Pharmacol Bull 1998;14:27–29
21. Ma LY, Xiao PG, Liang FQ, et al. Protective effects of *Panax* notoginseng saponins on primary cortical cultures of rat. Chinese Pharm J 1998;33:143–145
22. Ma LY, Xiao PG. Effects of PNS on synaptosomal glutamate release and on its specific binding to glutamatic receptor. Chinese Pharmacol Bull 1998;14:311–314

23. Yan Y, Zhang Z, Sun S. Protective effects of total saponin of *Panax* notoginseng and notoginsengsides on the hypoxia induced damage of cultured vascular endothelial cells in vitro. Chinese J Exp Trad Med Formulae 2002;8:34–37
24. Cai YL, Li HW. Review of pharmacology of sanchi. J Shanxi Coll Trad Chinese Med 2001;24:57–58
25. He X, Xing D, Yan B, Xiang N, Du L. Pharmacokinetics of notoginsengside R1, Rg1 in panax notoginseng saponins (PNS) in normal and cerebral ischemia-reperfusion rats. Pharmacol Clin Chinese Materia Medica 2001;17:12–14

58

Effect of Hydrocortisone on Excess Natriuresis in Patients with Aneurysmal Subarachnoid Hemorrhage

NOBUHIRO MORO, M.D., YOICHI KATAYAMA, M.D., PH.D., JUN KOJIMA, PH.D., TATSURO MORI, M.D., PH.D., TATSURO KAWAMATA, M.D., PH.D.

Abstract

Hyponatremia is commonly observed in patients with aneurysmal subarachnoid hemorrhage (SAH). These patients may suffer from excess natriuresis and diuresis, which leads to a decrease in total blood volume and eventually to increased risk of cerebral vasospasm. It may be difficult to maintain normal blood volume with sodium and fluid replacement. The present study examined the effects of hydrocortisone, which promotes sodium retention in the kidney, on hyponatremia and hypovolemia in patients with SAH. Twenty-eight patients admitted with SAH between October 1999 and July 2001 were included in this study. Patients underwent direct intracranial surgery and then were randomized into two groups. One group was treated with hydrocortisone (1200 mg/day for 10 days, $n = 14$), and the other group was not treated with hydrocortisone ($n = 14$). Both groups underwent hemodynamic therapy. End points included laboratory data and water balance. In addition, neurological deficit due to cerebral vasospasm and clinical outcome 6 months after SAH were evaluated. Hydrocortisone significantly attenuated natriuresis and maintained normal serum sodium level. Hydrocortisone also reduced urine volume as well as total infusion volume. Two patients in the control group were moderately disabled, as graded by the Glasgow outcome scale, as a result of cerebral vasospasm. Patients treated with hydrocortisone, on the other hand, all made good recoveries. There were no serious side effects attributed to hydrocortisone treatment. In conclusion, hydrocortisone facilitates managements of hyponatremia in patients with SAH by attenuation of natriuresis. Hydrocortisone makes volume expansion therapy more effective by reducing urine volume and infusion volume, and may have therapeutic potential to prevent cerebral vasospasm.

Hyponatremia is commonly observed in the clinical course of central nervous system diseases.[1] Ten to 34% of patients with subarachnoid hemorrhage (SAH) show signs of hyponatremia.[2] In these patients, hyponatremia generally is due to excess sodium excretion or natriuresis. It may be difficult to ameliorate the hyponatremia by sodium replacement because this may induce further sodium excretion.[3] Concomitant

TABLE 58–1 Clinical Characteristics of Patients

		Control	Hydrocortisone
N		14	14
Gender	Male	4	6
	Female	10	8
Age	Mean	52	51
	Range	30–72	32–75
Hunt and Kosnik grade			
	I	1	1
	II	8	10
	III	5	2
	IV	0	1
Ruptured aneurysm location			
	Anterior cerebral artery	5	7
	Middle cerebral artery	6	4
	Internal carotid artery	3	3

with sodium excretion and perhaps more detrimental to the patient is the increased urine volume that results from the sodium loss. This probably is an osmotic diuresis and can result in hypovolemia. Maintenance of normal or increased blood volume in patients with hyponatremia and hypovolemia after SAH can also be difficult even with aggressive hemodynamic therapy. We have reported previously the effects of fludrocortisone to activate sodium resorption in the kidneys and prevent natriuresis.[4] In the present study, we have examined the effect of hydrocortisone on natriuresis.

Material and Methods

Twenty-eight patients with SAH were analyzed. After surgery, patients were randomized to one of two groups: control (n = 14) or treatment with hydrocortisone, 1200 mg/day (n = 14). Baseline clinical information was collected on all patients, and both groups were managed with hemodynamic therapy.[5] The aim of hypervolemic therapy was to keep the water balance equal and the central venous pressure between 8 and 12 cm H_2O. Mean arterial blood pressure was maintained at a level ~15% higher than the original level. Sodium balance was calculated and sodium was replaced to keep the serum sodium level above 140 mEq/L. Serum electrolytes, urine electrolytes, plasma osmolality, and urine osmolality were measured every 14 days. In addition, neurological deficit due to cerebral vasospasm and clinical outcome 6 months after onset, based on the Glasgow outcome scale, were evaluated.[6]

Results

Baseline clinical information was similar between groups (Table 58–1). Serum sodium concentration in the control group gradually decreased to < 140 mEq/L whereas it was maintained within the target range in patients treated with hydrocortisone [$p < .05$, analysis of variance (ANOVA), Fig. 58–1]. Sodium excretion increased in control patients and eventually exceeded 1200 mEq/day In the group treated with hydrocortisone, however, sodium excretion never exceeded 700 mEq/day ($p < .05$, ANOVA). Potassium excretion increased in the hydrocortisone group. The urinary sodium:potassium ratio was significantly higher in the control group compared with the hydrocortisone patients ($p < .05$, ANOVA, Fig. 58–2).

With regard to water, sodium balance, and urine volume, the control group required large volumes of

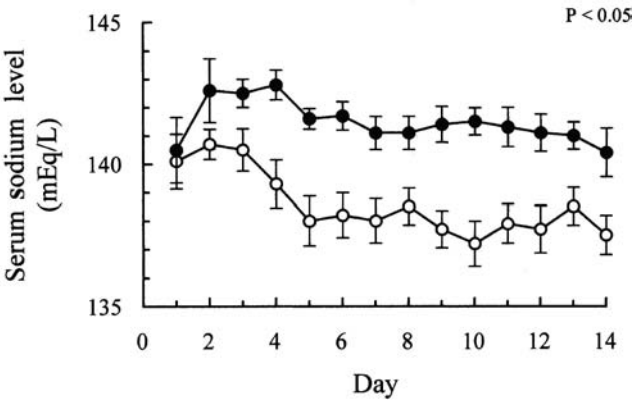


FIGURE 58–1 Serum sodium levels, by day, in control patients (open circles) and patients treated with hydrocortisone (closed circles). The mean serum sodium level decreased to < 140 mEq/L in control patients. In contrast, the mean sodium level was maintained at > 140 mEq/L throughout the period of observation in patients treated with hydrocortisone. Values are expressed as means ± standard error of the mean ($p < .05$, ANOVA).

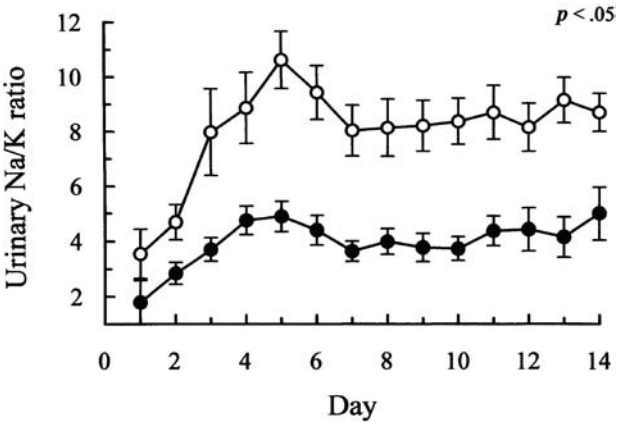


FIGURE 58–2 Urinary sodium:potassium ratio for each day in control patients (open circles) and hydrocortisone-treated patients (closed circles). The urinary sodium:potassium ratio was markedly elevated in control patients compared with those treated with hydrocortisone. Values are means ± standard error of the mean ($p < .05$, ANOVA).

fluid and sodium replacement to maintain water and sodium balance. The urine volume exceeded 8 L/day. Hydrocortisone treatment reduced urine volume and effectively prevented a negative shift in sodium as well as in the water balance.

Two patients in the control group were moderately disabled, as graded by the Glasgow outcome scale, as a result of cerebral vasospasm. Patients treated with hydrocortisone, on the other hand, all made good recoveries. There were no serious side effects attributed to hydrocortisone treatment.

Discussion

The present results demonstrate that patients with aneurysmal SAH (the control group in this study) develop natriuresis and increased urine output, probably reflecting an osmotic diuresis. There was a close correlation between the urine volume and the sodium excretion. During the acute stage after SAH, patients with natriuresis required a large amount of fluid and sodium replacement to maintain an appropriate blood volume. Natriuresis and the diuresis that follows accounted for cerebral salt wasting syndrome. It was difficult to maintain the serum sodium level even with aggressive sodium administration. Hydrocortisone is known to promote sodium resorption in the kidneys and the present results suggest that it can attenuate the excessive natriuresis that occurs after SAH. It can also facilitate hemodynamic therapy by allowing one to more readily achieve induced hypervolemia. This may contribute to prevention of symptomatia cerebral vasospasm. Hydrocortisone may come to play an important role in the management of patients with SAH and particularly in those in whom hemodynamic therapy is utilized.

Conclusion

Hydrocortisone may be useful in the management of patients after SAH because it inhibits natriuresis and reduces urine volume as well as infusion volumes required to maintain sodium and water balance. Hydrocortisone may have therapeutic potential in the prevention of cerebral vasospasm after SAH.

REFERENCES

1. Harrigan MR. Cerebral salt wasting syndrome. Crit Care Clin 2001;17:125–138
2. Mayberg MR, Batjer HH, Dacey R, et al. Guidelines for the management of aneurysmal subarachnoid hemorrhage: a statement for healthcare professionals from a special writing group of the stroke council, American Heart Association. Stroke 1994;25: 2315–2328
3. Raymond KH, Reineck HJ, Stein JH. Sodium metabolism and maintenance of extracellular fluid volume. In: Arieff AI, DeFronzo RA, eds. Fluid Electrolyte and Acid-Base Disorders. Vol 1. New York: Churchill Livingstone; 1985:39–76
4. Mori T, Katayama Y, Kawamata T, Hirayama T. Improved efficiency of hypervolemic therapy with inhibition of natriuresis by fludrocortisone in patients with aneurysmal subarachnoid hemorrhage. I Neurosurg 1999;91:947–952
5. Hunt WE, Kosnik EJ. Timing and perioperative care in intracranial aneurysm surgery. Clin Neurosurg 1974;21:79–89
6. Jennett B, Bond M. Assessment of outcome after severe brain damage: a practical scale. Lancet 1975;1:480–484

59

Safety and Efficacy of the Different Components of Hemodynamic Therapy in Patients with Symptomatic Vasospasm

ANDREAS RAABE, M.D., PH.D., JÜRGEN BECK, M.D., HARTMUT VATTER, M.D., MICHAEL ZIMMERMANN, M.D., PH.D., VOLKER SEIFERT, M.D., PH.D.

Abstract

Hypertensive hypervolemic hemodilution or hemodynamic therapy is used routinely for prophylaxis and treatment of symptomatic cerebral vasospasm in many institutions. However, there is an ongoing debate about the indications (prophylactic or therapeutic), the modality (hypervolemia, hypertension, or both), the therapy intensity (moderate or aggressive), and the risks and benefits of this therapy in patients with subarachnoid hemorrhage (SAH). Monitoring data and medical records of 45 patients were retrospectively searched to identify periods of hypervolemia, moderate hypertension, or aggressive hypertension. Measurements of central venous pressure, fluid input, urine output, arterial blood pressure, intracranial pressure, and brain tissue oxygen were extracted from periods ranging from 1 hour to 24 hours. For these periods, the change in cerebral oxygenation was analyzed. Side effects and complications that occurred during these treatment periods were registered from the patient daily assessment and complications record. During the 55 periods of moderate hypertension, an increase in brain oxygenation was found in 50 cases (90%). Complications of moderate hypertension were observed in three patients (8%). During the 25 periods of hypervolemia, an increase in brain oxygenation was found during three periods (12%). Complications of hypervolemia were observed in nine patients (53%) with multiple complications in six cases. During the 10 periods of aggressive hypervolemic hypertension, an increase in brain oxygenation was found during six (60%). Complications of hypervolemia were observed in five patients (50%) with multiple complications in all of these cases. Vigorous treatment of hypovolemia and hypotension remains the mainstay of fluid and pressure management after SAH. In normovolemic patients, hypervolemia without increase in arterial pressure and cerebral perfusion pressure does not improve cerebral oxygenation and has a low benefit:risk ratio with increasing therapy intensity. Moderate hypertension (cerebral perfusion pressure of 80–120 mmHg) is effective in improving cerebral oxygenation with a very low complication rate and should be the mainstay in the treatment of symptomatic vasospasm.

Hypertensive hypervolemic hemodilution or hemodynamic therapy is used routinely for prophylaxis and treatment of symptomatic cerebral vasospasm in many institutions. However, there is an ongoing debate about the indications (prophylactic or therapeutic), the modality (hypervolemia, hypertension, or both), the therapy intensity (moderate or aggressive), and the risks and benefits of hemodynamic therapy in patients with subarachnoid hemorrhage (SAH).[1,2] There are no data available on whether the different treatment modalities and intensity levels have a comparable effect on cerebral circulation and a similar complication rate. However, this data would be of clinical importance because hemodynamic therapy is used currently in a variety of ways, and the effectiveness of this therapy has been questioned.[1] Moreover, hemodynamic therapy is a potentially harmful treatment and is associated with complications in up to 20 to 30% of cases.[3–8]

This study investigated the effects of three different forms of therapeutic hemodynamic therapy on brain tissue oxygenation in patients with SAH and angiographically proven cerebral vasospasm.

Material and Methods

Study Population

Invasive monitoring of brain tissue oxygenation was performed in patients who were not able to cooperate for adequate neurological assessment. This included patients in poor clinical grades (Hunt and Hess grade 4)[9] or those patients who were grades 1 to 3 but where intubation, analgesics, and sedation were required for management of either or both complications and clinical deterioration. A tissue oxygen probe was inserted either after aneurysm clipping or at the time of clinical deterioration and intubation of the patient. Exclusion criteria for hemodynamic therapy were established cerebral infarction, transtentorial herniation, intracerebral hemorrhage, brain edema causing medically refractory increases in intracranial pressure to > 25 mmHg, pulmonary edema, congestive heart failure, and myocardial infarction. Patients with technical difficulties related to the brain tissue probe were also excluded. Data were collected prospectively from 45 patients with reliable tissue oxygen measurements.

Fluid and Pressure Management

After admission, a central venous catheter was placed in all patients and hypovolemia, hyponatremia, and hypotension were treated irrespective of whether or not the aneurysm was secured. Target values were cerebral perfusion pressure > 60 mmHg, central venous pressure (CVP) > 4 mmHg, and hematocrit < 0.45. After confirmation of clinically relevant vasospasm, hypervolemia or hypertension were instituted. The first step of hemodynamic therapy was usually induced hypertension to achieve a cerebral perfusion pressure of > 80 mmHg but < 120 mmHg. We did not include hypervolemia in this step because we believe that a rather high-normal normovolemia (CVP ≥ 5 mmHg) provides an acceptable intravascular volume state for administration of vasopressors. Induced hypertension was started with dopamine, 5 to 12 µg/kg/min and, if necessary, supplementary norepinephrine, 0.1 to 0.5 µg/kg/min, to maintain the mean cerebral perfusion pressure between 80 and 120 mmHg.

Alternatively, hypervolemia was the first step of hemodynamic therapy in those patients who already had a cerebral perfusion pressure between 80 and 120 mmHg or in those who did not respond to vasopressors when attempts were made to induce moderate hypertension. The target of hypervolemia was a CVP of > 10 mmHg, and this was achieved by infusion of crystalloids, hydroxy ethyl starch up to a maximum of 1000 mL per day, erythrocytes, and albumin. Failure of these steps to improve clinical vasospasm, tissue oxygenation, or somatosensory evoked potential amplitude/latency resulted in commencing aggressive induced hypertension. This treatment consisted of increasing the dose of vasopressors to raise the mean cerebral perfusion pressure > 120 mmHg. Systolic pressures of > 220 mmHg were avoided.

Data Acquisition

Monitoring of brain oxygenation was performed using a rexovode tissue oxygen probe (Licox GMS, Kiel, Germany) implanted in the territory of the parent artery harboring the ruptured aneurysm. For the middle cerebral artery, this was located 1 cm in front of the coronal suture and 6 cm off the midline. For the anterior cerebral artery, the location was 1 cm in front of the coronal suture and 2 cm off the midline. Real-time analog signals from tissue oxygen monitoring, arterial blood pressure, and intracranial pressure were collected and averaged every 2 seconds and digitized using an A to D converter card. A multimodal monitoring software (BioSAn, Biological Signal Analyzer, P. Smielewski, M. Czosnyka, Cambridge, UK) was used for data storage and online or offline analysis.

Data Analysis and Statistics

Monitoring data and patient charts were retrospectively searched to identify the point of time when moderate hypertension, aggressive hypertension, or hypervolemia were begun. Measurements of CVP, fluid input, urine output, arterial blood pressure, intracranial pressure, and brain tissue oxygen were extracted from

periods ranging from 1 hour to 24 hours. For these periods, the change in cerebral oxygenation was analyzed during (1) moderate cerebral perfusion pressure increase (moderate hypertension), (2) further increase in cerebral perfusion pressure (aggressive hypertension), and (3) hypervolemic treatment. Side effects and complications that occurred during these treatment periods were obtained from the patient daily assessments and problems or complications record.

The association between continuous variables was analyzed using Pearson's correlation coefficient. Continuous variables were compared using two-tailed *t*-tests and proportions were compared using the x^2 or Fisher's exact test. Significance was judged at the $p < .05$ level.

Results

A total of 90 periods of induced moderate hypertension, aggressive hypertension, or hypervolemia were analyzed in 45 patients. We identified 55 periods of moderate hypervolemic hypertension, 10 periods of aggressive hypertensive, hypervolemic therapy, and 25 periods of hypervolemia. During the *55* periods of moderate hypertension (38 patients, one–three periods per patient), an increase in brain oxygenation (median 6 mmHg, interquartile range 3–10 mmHg) was found in 50 cases (90%). Complications of moderate hypertension were observed in three patients (8%). These included hyponatremia in one patient (3%) and cardiac arrhythmia in two patients (5%).

During the 25 periods of hypervolemia (17 patients, 1–2 periods per patient), an increase in brain oxygenation was found during three periods (12%). Complications of hypervolemia were observed in nine patients (53%), with multiple complications in six cases. Pulmonary fluid overload occurred during eight periods of hypervolemia, pulmonary edema during three, hyponatremia during three, cardiac arrhythmia during three, congestive heart failure during two, and brain edema during two.

During the 10 periods of aggressive hypervolemic hypertension (10 patients, one period per patient), an increase in brain oxygenation was found during six periods (60%). Complications of hypervolemia were observed in five patients (50%) with multiple complications in all cases. Pulmonary fluid overload occurred in two patients, pulmonary edema in one, hyponatremia in one, cardiac arrhythmia in two, sepsis in three, and brain edema in two.

Discussion

The effects of hypervolemic therapy on cerebral blood flow in patients with SAH have been inconsistent in the literature. Origitano et al reported an increase in cerebral blood flow when hypervolemic therapy was instituted shortly after admission for SAH. They did not, however, measure baseline volume status of their patients and it is possible that the patients were hypovolemic before treatment.[10] Another report suggesting that hypervolemia increases cerebral blood flow also included hypovolemic patients as shown by low normal pulmonary artery wedge pressures and low cardiac outputs.[11]

Yamakami et al performed 55 pairs of regional cerebral blood flow measurements using the xenon-133 inhalation method before and after volume expansion in 35 patients with ruptured cerebral aneurysms. After volume expansion with albumin, the hemoglobin value decreased significantly. Volume expansion did not change the mean arterial blood pressure. Measurement of cerebral blood flow after volume expansion showed a decrease in patients with symptomatic vasospasm and no change in patients without symptomatic vasospasm.[12] In a recent prospective study, 82 patients were randomly assigned to receive either hypervolemic or normovolemic treatment up to day 14 after SAH and aneurysm surgery.[13] Volume expansion was guided using pulmonary artery diastolic pressure for the first 3 days after SAH and thereafter using CVP. Cerebral blood flow was measured before randomization and every 3 days thereafter using the ^{133}xenon clearance method. The authors found no difference between the groups in global and minimum local cerebral blood flow during the treatment period. Cerebral infarction occurred in 17% of hypervolemic patients and in 10% of normovolemic patients. The authors concluded that prophylactic hypervolemic therapy was unlikely to confer an additional benefit. In conclusion, there is lack of data supporting the use of hypervolemia in normovolemic patients.

Adverse effects most often reported in patients treated with hemodynamic therapy include fluid overload (10–40%), pulmonary edema (2–34%), congestive heart failure (5–20%), cardiac arrhythmias (5%), and aggravation of brain edema (8–20%).[3–8] Numerous other complications are reported including hypertensive encephalopathy, hemorrhagic infarction, hyponatremia, rebleeding from the aneurysm, coagulopathy, myocardial infarction, hemothorax, and intra-abdominal bleeding.[5,14–17] There is a clear relationship between therapy intensity and the occurrence of adverse effects.

Conclusion

Vigorous treatment of hypovolemia and hypotension remains the mainstay of fluid and blood pressure management after SAH. Our results and those reviewed

from the literature suggest that in normovolemic patients, hypervolemia without an increase in arterial pressure and cerebral perfusion pressure does not improve cerebral oxygenation and has a low benefit:risk ratio that worsens as the intensity of therapy increases. Therefore, it cannot be recommended either as a routine measure after surgery or as a treatment for symptomatic vasospasm. There is no additional role for fluid therapy besides providing optimum vascular filling to allow effective use of vasopressors. Moderate hypertension (cerebral perfusion pressure 80–120 mmHg) is effective in improving cerebral oxygenation with a very low complication rate and should be the mainstay in the treatment of symptomatic vasospasm. Aggressive hypertension (cerebral perfusion pressure > 120 mmHg) may further improve cerebral oxygenation; however, there is an increasing risk of side effects and a decreasing benefit with increasing therapy intensity.

REFERENCES

1. Oropello JM, Weiner L, Benjamin E. Hypertensive, hypervolemic, hemodilutional therapy for aneurysmal subarachnoid hemorrhage: is it efficacious? No. Crit Care Clin 1996;12:709–730
2. Ullman JS, Bederson JB. Hypertensive, hypervolemic, hemodilutional therapy for aneurysmal subarachnoid hemorrhage: is it efficacious? Yes. Crit Care Clin 1996;12:697–707
3. Hadeishi H, Mizuno M, Suzuki A, Yasui N. Hyperdynamic therapy for cerebral vasospasm. Neurol Med Chir (Tokyo) 1990;30:317–323
4. Haraguchi S, Ebina K. Evaluation of the dopamine induced hypertension therapy for vasospasm. No Shinkei Geka 1982;10:279–289
5. Kassell NF, Peerless SJ, Durward QJ, et al. Treatment of ischemic deficits from vasospasm with intravascular volume expansion and induced arterial hypertension. Neurosurgery 1982;11:337–343
6. Medlock MD, Dulebohn SC, Elwood PW. Prophylactic hypervolemia without calcium channel blockers in early aneurysm surgery. Neurosurgery 1992;30:12–16
7. Shimoda M, Oda S, Tsugane R, Sato O. Intracranial complications of hypervolemic therapy in patients with a delayed ischemic deficit attributed to vasospasm. J Neurosurg 1993;78:423–429
8. Diringer MN, Wu KC, Verbalis JG, Hartley DF. Hypervolemic therapy prevents volume contraction but not hyponatremia following subarachnoid hemorrhage. Ann Neurol 1992;31:543–550
9. Hunt WE, Hess RM. Surgical risk as related to time of intervention in the repair of intracranial aneurysms. J Neurosurg 1968;28:14–20
10. Origitano TC, Wascher TM, Reichman OH, Anderson DE. Sustained increased cerebral blood flow with prophylactic hypertensive hypervolemic hemodilution ("triple-H" therapy) after subarachnoid hemorrhage. Neurosurgery 1990;27:729–739
11. Mori K, Arai H, Nakajima K, et al. Hemorheological and hemodynamic analysis of hypervolemic hemodilution therapy for cerebral vasospasm after aneurysmal subarachnoid hemorrhage. Stroke 1995;26:1620–1626
12. Yamakami I, Isobe K, Yamaura A. Effects of intravascular volume expansion on cerebral blood flow in patients with ruptured cerebral aneurysms. Neurosurgery 1987;21:303–309
13. Lennihan L, Mayer SA, Fink ME, et al. Effect of hypervolemic therapy on cerebral blood flow after subarachnoid hemorrhage: a randomized controlled trial. Stroke 2000;31:383–391
14. Amin-Hanjani S, Schwartz RB, Sathi S, Stieg PE. Hypertensive encephalopathy as a complication of hyperdynamic therapy for vasospasm: report of two cases. Neurosurgery 1999;44:1113–1116
15. Awad IA, Carter LP, Spetzler RF, et al. Clinical vasospasm after subarachnoid hemorrhage: response to hypervolemic hemodilution and arterial hypertension. Stroke 1987;18:365–372
16. Otsubo H, Takemae T, Inoue T, et al. Normovolaemic induced hypertension therapy for cerebral vasospasm after subarachnoid haemorrhage. Acta Neurochir (Wien) 1990;103:18–26
17. Rassias AJ, Harbaugh RE, Corwin HL. Intra-abdominal hemorrhage complicating hypertensive therapy for cerebral vasospasm. Crit Care Med 1995;23:775–777

SECTION IX

Clinical—Surgery and Endovascular

60

Symptomatic Vasospasm After Early Surgical and Endovascular Treatment of Ruptured Cerebral Aneurysms

MASARU HIROHATA, M.D., NAOKO FUJIMURA, M.D., YASUHARA TAKEUCHI, M.D., TAKASHI TOKUTOMI, M.D., MINORU SHIGEMORI, M.D.

Abstract

The influence of the treatment modality (surgery vs endovascular treatment) on the incidence of cerebral vasospasm was studied in a nonrandomized series of 245 consecutive patients treated within 72 hours of aneurysmal subarachnoid hemorrhage (SAH) between 1998 and 2001. The 76 patients treated with surgery had a mean age of 63 and included 17 males and 59 females. Hunt and Hess grades were grade 2 in 43, 3 in 24, and 4 in nine patients. Endovascular treatment was employed in 137 patients with a mean age of 60 (46 males and 91 females). Hunt and Hess grades were grade 2 in 63, 3 in 50, and 4 in 24 patients. The age and Hunt and Hess grades were not significantly different between the two groups. Postoperative adjuvant therapies for prevention of vasospasm were basically the same in both groups. Symptomatic vasospasm occurred in 21 (28%) patients undergoing surgery and 8 (6%) patients treated endovascularly. Permanent neurological deficit due to vasospasm occurred in 12 (16%) surgical and 4 (3%) endovascular patients. The incidences of symptomatic vasospasm ($p < .001$) and permanent neurological deficit due to vasospasm ($p = .002$) were significantly lower in the endovascular compared with the surgery group.

The International Subarachnoid Aneurysm Trial reported that the clinical outcome was better for ruptured cerebral aneurysms treated with endovascular coil embolization than for those treated with neck clipping.[1] However, the influence of the treatment modality on the incidence of symptomatic vasospasm was not and has not been fully characterized after treatment with these two modalities.[2–4] Therefore, the objective of the present study was to analyze the relationship between the treatment modality that was applied and the incidence of cerebral vasospasm in a nonrandomized series of patients with aneurysmal subarachnoid hemorrhage (SAH).

Patients and Methods

From 1998 to 2002, 245 patients with ruptured cerebral aneurysms were admitted to and treated at Kurume University hospital within 72 hours of the ictus. Among these patients, 32 had intracerebral hematomas. Most of the patients with intracerebral hemorrhage had focal neurological deficits making a diagnosis of delayed

TABLE 60–1 Clinical Data on 213 Patients with Aneurysmal Subarachnoid Hemorrhage

	Coil Embolization (*n* == 137)	Surgical Clipping (*n* = 76)	*p* Value
Age	**60** ±13	**63** ±13	**.07**
Sex			**.08**
Male	**46**	**17**	
Female	**91**	**59**	
Hunt and Hess grade			**.36**
2	**63**	**43**	
3	**50**	**24**	
4	**24**	**9**	
Fisher grade			**.34**
2	**25**	**18**	
3	**112**	**58**	
Aneurysm location			**<.001**
Anterior	**104**	**74**	
Posterior	**33**	**2**	

ischemic neurological deficit (symptomatic vasospasm) due to cerebral vasospasm difficult. Therefore, patients with intracerebral hemorrhage were excluded from this study. The remaining 213 patients consisted of 150 females and 63 males and were included in this study. Patients were managed according to the following protocol. The primary treatment recommendation was endovascular packing with Guglielmi detachable coils. Surgical clipping was recommended for patients with an aneurysm of unsuitable form or size for coiling. All patients were managed in the neurosurgical intensive care unit. Both therapeutic groups were subjected to the same postoperative treatment. All patients received intravenous fluids to maintain normovolemia and received ozagrel sodium and fasudil hydrochloride hydrate routinely.[5] Patients treated endovascularly underwent spinal cerebrospinal fluid drainage, and patients treated by surgery had cisternal drainage. The purpose of cerebrospinal fluid drainage was to drain bloody cerebrospinal fluid. The incidence of symptomatic vasospasm and the outcome at discharge were compared between the two groups using the x^2 test.

Results

137 patients (64%) were treated with coil embolization and 76 patients were treated with surgery. There were no statistically significant differences between the two groups in terms of age, sex, Hunt and Hess grade,[6] and Fisher computed tomographic (CT) grade.[7] The percentage of patients with posterior circulation aneurysms was significantly higher in the group treated with coils compared with those treated with surgery ($p < .001$, Table 60–1). Thirty-four patients (16% of all patients) suffered from delayed ischemic neurological deficits or symptomatic vasospasm during their hospitalization. The incidence of symptomatic vasospasm was positively correlated with the initial Fisher grade (Table 60–2). Six percent (8/137) of patients who were treated with endovascular coils developed symptomatic vasospasm. This was significantly lower than the number of patients (28%, 21/76) who were treated by surgical clipping and who developed symptomatic vasospasm ($p < .001$). Five of the eight patients with symptomatic vasospasm in the endovascular group and 8 of the 21 with symptomatic vasospasm in the surgery group were treated with a super-selective intraarterial infusion of papaverine hydrochloride or fasudil hydrochloride hydrate. Permanent ischemic neurological deficits from vasospasm occurred in four (3%) of the patients treated with coils and 12 (16%) of the patients treated with surgery ($p = .006$, Table 60–3).

Discussion

The reported incidence of symptomatic vasospasm after aneurysmal SAH treated with surgery was 34%.[5] Recently, various adjuvant therapies for the prevention

TABLE 60–2 Relationship between Fisher Grade and Incidence of Symptomatic Vasospasm

Fisher grade	2 (*n* = 43)	3 (*n* = 170)	*P* Value
Symptomatic vasospasm	2 (5%)	32 (19%)	0.023

TABLE 60–3 Relationship between Hunt and Hess Grade and Incidence of Permanent Delayed Ischemic Neurological Deficit

	Hunt and Hess Grade			
Treatment	2	**3**	**4**	**Total**
Coil embolization	1/63(1.5%)	1/50(2%)	2/24 (8%)	4/137 (3%)
Surgical clipping	7/43 (16%)	3/27 (11%)	2/9 (22%)	12/76 (16%)
p Value	.005	.085	.22	.006

of symptomatic vasospasm have been developed including intravenous infusion of either or both fasudil hydrochloride and ozagrel sodium[5] and cisternal irrigation.[3] These treatments are able to reduce the incidence of symptomatic vasospasm. Nevertheless, other investigators have reported that ~30% of patients treated with aneurysmal neck clipping still suffer from symptomatic vasospasm.[3] Symptomatic vasospasm remains a major cause of morbidity and mortality in patients with SAH.

The reported incidence of symptomatic vasospasm after treatment with coils has ranged from 14 to 30%.[2–4] This incidence seems to be equal to or lower than that reported in patients treated with surgery. These reports themselves, however, are not comparable amongst each other due to differences in the patient populations, and the studies were not randomized so that numerous factors could account for the differences, not the least of which being the mode of aneurysm treatment. Gruber et al compared the incidence of vasospasm in patients treated with coils or surgery in one institution and concluded that the incidence was higher in patients treated with coils (16% vs 12%).[2] However, Debrun and colleagues reported the opposite results.[4] In their series, symptomatic vasospasm was significantly less frequent in patients treated by coiling compared with those undergoing surgery (20% vs 43%). Charpentier et al also reported less symptomatic vasospasm in endovascularly treated patients (17%) compared with surgical patients (22%).[8]

The present series collected patients over the past 5 years. Patient age, sex, Hunt and Hess grade, and Fisher grade were not significantly different between our patients treated with surgery and those with coiling. Although the location of the aneurysms differed significantly between the treatment groups, there is no definitive evidence that the aneurysm location influences the risk of symptomatic vasospasm.[3] The incidence of permanent deficits from vasospasm was lower in the coil embolized patients than in clipped patients (3% vs 16%). The reason for this difference is not clear, but several hypotheses have been put forward. The most accepted explanation for the development of vasospasm is the presence of blood in the subarachnoid space and of degradation products of the blood, including hemoglobin, surrounding the cerebral arteries. Although early clot removal by surgery has been reported to decrease vasospasm, several clinical studies failed to provide evidence for benefit of this procedure.[3,8] Moreover, surgical mechanical manipulation may cause vasospasm, at least transiently.[8] Although coil embolization would not be expected to remove the subarachnoid clot, in the present series, lumbar spinal fluid drainage may have helped wash out the perivascular clot. Coil embolization also has advantages of being less invasive. It involves less mechanical manipulation of the cerebral vessels. The limited invasiveness of this procedure may be a reasonable explanation for the low incidence of symptomatic embolization after coil embolization.

REFERENCES

1. Molyneux A, Kerr R, Stratton I, et al. International Subarachnoid Aneurysmal Trial (ISAT) of neurosurgical clipping versus endovascular coiling in 2143 patients with ruptured intracranial aneurysms: a randomised trial Lancet 2002;360:1267–1274
2. Gruber A, Ungersbock K, Reinprecht A, et al. Evaluation of cerebral vasospasm after early surgical and endovascular treatment of ruptured intracranial aneurysms. Neurosurgery 1998;42:258–268
3. Rabinstein AA, Pichelmann MA, Friedman J, et al. Symptomatic vasospasm and outcome following aneurysmal subarachnoid hemorrhage: a comparison between surgical repair and endovascular coil occlusion. J Neurosurg 2003;98:319–325
4. Debrun GM, Aletich VA, Kehrli P, et al. Evaluation of cerebral vasospasm after early surgical and endovascular treatment of ruptured intracranial aneurysms (letter). Neurosurgery 2001;49: 646–647
5. Nakashima S, Tabuchi K, Shimokawa S, Fukuyama K, Mineta T, Abe M. Combination therapy of fasudil hydrochloride and ozagrel sodium for cerebral vasospasm following aneurysmal subarachnoid hemorrhage. Neurol Med Chir (Tokyo) 1998;38:805–811
6. Hunt WE, Hess RM. Surgical risk as related to time of intervention in the repair of intracranial aneurysms. J Neurosurg 1968;28:14–20
7. Fisher CM, Kistler JP, Davis JM. Relation of cerebral vasospasm to subarachnoid hemorrhage visualized by computerized tomographic scanning. Neurosurgery 1980;6:1–9
8. Charpentier C, Audibert G, Guillemin F, et al. Multivariate analysis of predictors of cerebral vasospasm occurrence after aneurysmal subarachnoid hemorrhage. Stroke 1999;30:1402–1408

61

Endovascular Treatment for Cerebral Vasospasm

YOSHIHIRO TAKANASHI, M.D., PH.D., SATOSHI FUJII, M.D., JUNICHI UMEKAWA, M.D., TOSHIYUKI YOSHIDA, M.D.

Abstract

Endovascular treatment for vasospasm is usually reserved for patients who continue to exhibit clinical deterioration despite maximal medical therapy. This study evaluated the effects at a single institution of endovascular therapy on vasospastic cerebral vessels. We retrospectively reviewed the medical records of 180 consecutive patients with ruptured intracranial aneurysms who were hospitalized between August 1999 and January 2003. After definitive therapy for the ruptured aneurysm, each patient was treated according to standard intensive care protocols before and after endovascular therapy. Cerebral perfusion and the general hemodynamic status of each patient were maintained in a hypervolemic condition. Patients who developed signs of clinical deterioration or significant abnormalities on transcranial Doppler (TCD) studies despite the best medical treatment were included in the current study. There were 41 patients who developed clinical cerebral vasospasm. Of these, 16 patients fulfilled the aforementioned criteria and were treated with either papaverine infusion with balloon angioplasty (n = 2) or drug infusion alone (n = 14). There was no correlation between TCD velocities and clinical outcome as assessed by the Glasgow outcome scale. Overall, 11 patients (68%) experienced a favorable outcome at 6-month follow-up examination. One patient had vessel rupture during inflation of the microballoon. We suggest that endovascular strategies for treatment of vasospasm might be considered as soon as possible after neurological decline for any patient who is unresponsive to medical therapy However, we observed that in this small series, angiographic improvement and neurological amelioration do not necessarily correspond.

Cerebral vasospasm due to subarachnoid hemorrhage (SAH) remains a leading treatable cause of morbidity and mortality in patients with ruptured intracranial aneurysms. Endovascular treatment with balloon angioplasty and intra-arterial drug infusion is often reserved for patients who continue to exhibit clinical deterioration despite maximal medical therapy.[1] However, the clinical efficacy of endovascular treatment for patients who develop symptomatic vasospasm after aneurysmal SAH is still unclear. It has been reported that angiographic improvement and neurological amelioration do not necessarily correspond.[2] Moreover, the current reported rates of serious complications associated with endovascular treatment are in the range of 2 to 5%.[3] This retrospective review was performed to assess the clinical efficacy of balloon angioplasty and intra-arterial drug infusion for treatment of cerebral vasospasm following SAH at a single institution.

Patients and Methods

We retrospectively reviewed the medical records of 180 consecutive patients with ruptured intracranial aneurysms who were treated at the Yokohama Stroke and Brain Center, Yokohama, Japan, between August 1999 and January 2003. After definitive therapy of the ruptured aneurysm, each patient was treated according to standard intensive care protocols before and after endovascular therapy. Cerebral perfusion was maintained with the help of induced hypervolemia. Patients who had signs of clinical deterioration or significant abnormalities on transcranial Doppler (TCD) studies despite best medical treatment were included in the current study. Endovascular treatment was employed in these patients if they developed a mean middle cerebral artery blood flow velocity on TCD of > 120 cm/sec and/or there was an increase in flow velocity in this artery of > 50 cm/sec in 24 hours. Patients were also included only if a cranial computed tomographic scan did not show any low-density areas and there was 30% or more stenosis of a major cerebral artery on a cerebral angiogram. All endovascular procedures were performed via the transfemoral approach. Intra-arterial drug infusion was performed through a microcatheter with its tip placed above the origin of the ophthalmic artery. The main indication for balloon angioplasty was angiographic evidence of proximal arterial vasospasm. Cerebral angiograms were obtained and measurements of arterial diameters were performed to determine whether there were changes in the diameters of spastic intracranial arteries after endovascular therapy.

Results

There were 41 patients who developed clinically evident cerebral vasospasm. The ruptured aneurysms were secured in all patients by surgical obliteration. Hunt and Hess grades on admission are summarized in Table 61–1.[4] Of these, 16 patients fulfilled the aforementioned criteria and were treated with either papaverine infusion plus balloon angioplasty (n = 2) or drug infusion alone (n = 14). The largest numbers of endovascular treatments were performed 8 to 10 days after SAH. The majority of the aneurysms were located in the anterior circulation (n = 14, 87%). There was no correlation between TCD velocities and clinical outcome as assessed on the Glasgow outcome scale.[5] Twenty-six of 45 vessel segments (57%) showed angiographic improvement after endovascular treatment. Resolution of neurological deficits occurred in nine patients (56%). Overall, 11 patients (68%) experienced a favorable outcome at 6-month follow-up examination (Table 61–2). One patient had vessel rupture during inflation of the microballoon.

TABLE 61–1 Clinical Characteristics of 41 Patients with Symptomatic Vasospasm

Parameter		Number of Cases (%)
Hunt and Hess grade	1	**13 (32%)**
	2	**5 (12%)**
	3	**11 (27%)**
	4	**9 (22%)**
	5	**3 (7%)**
Glasgow outcome scale	Good recovery	**18 (44%)**
	Moderate disability	4(10%)
	Severe disability	**7 (17%)**
	Vegetative	**5 (12%)**
	Dead	**7 (17%)**

Discussion

It has been reported that selective intra-arterial drug infusion and balloon angioplasty are effective in the treatment of cerebral vasospasm following SAH.[6] Delays in instituting therapy for cerebral vasospasm can lead to irreversible cerebral infarction and a devastating outcome. This may be one explanation for the reports that endovascular treatments occasionally seem to have no significant impact on patient outcome.[7,8] Furthermore, there are still significant risks associated with endovascular procedures, such as vessel occlusion, thrombus formation, and even vessel rupture during inflation of the microballoon.[3]

Intra-arterial drug infusion can be useful as an adjunct to balloon angioplasty and also for the treatment of distal vessels that are not accessible to balloon

TABLE 61–2 Clinical Characteristics of 16 Patients Treated with Balloon Angioplasty or Intra-Arterial Drug Infusion or Both

Parameter		Number of Cases (%)
Hunt and Hess grade	1	**7 (44%)**
	3	**3 (19%)**
	3	**2 (13%)**
	4	**4 (25%)**
	5	**0 (0%)**
Glasgow outcome scale	Good recovery	**8 (50%)**
	Moderate disability	**3 (19%)**
	Severe disability	**3 (19%)**
	Vegetative	**0 (0%)**
	Dead	**2 (13%)**

angioplasty. However, it has been reported that intra-arterial drug infusion can be associated with adverse effects.[9–12] Therefore, most authorities recommend that there should be a firm indication for endovascular treatment for cerebral vasospasm as opposed to treatment of angiographic vasospasm alone.

Oskouian et al retrospectively analyzed the effects of balloon angioplasty, intra-arterially administered papaverine, and the combination on hemispheric cerebral blood flow, TCD velocities, and cerebral artery diameters in 45 patients with aneurysmal SAH.[2] Endovascular treatment for cerebral vasospasm was associated with significant and sustained increases in cerebral blood flow and vessel diameters as well as significant decreases in TCD velocities. Twenty-six of 45 vessel segments (57%) improved angiographically after endovascular treatment, and neurological deficits resolved in nine patients (20%). These changes, however, did not result in differences in overall outcomes. These results show that angiographic and clinical improvement do not necessarily correspond. Because timely intervention is often overlooked, it is important that endovascular treatment for cerebral vasospasm be considered as soon as possible after neurological decline for patients who are refractory to best medical therapy.

In summary, endovascular treatment with balloon angioplasty and intra-arterial drug infusion for cerebral vasospasm following SAH is often useful for patients who continue to exhibit clinical deterioration despite maximal medical therapy. However, we observed that angiographic improvement and amelioration of neurological deficits do not necessarily correspond. In terms of endovascular treatment for cerebral vasospasm, prospective clinical trials will be required for delineation of the precise role of these procedures.

REFERENCES

1. Newell DW, Elliott JP, Eskridge JM, et al. Endovascular therapy for aneurysmal vasospasm. Crit Care Clin 1999;15:685–699
2. Oskouian RJ, Martin NA, Lee JH, et al. Multimodal quantitation of the effects of endovascular therapy for vasospasm on cerebral blood flow, transcranial Doppler ultrasonographic velocities, and cerebral artery diameters. Neurosurgery 2002;51:30–43
3. Eskridge JM, Song JK. A practical approach to the treatment of vasospasm. AJNR Am J Neuroradiol 1997;18:1653–1660
4. Hunt WE, Hess RM. Surgical risk as related to time of intervention in the repair of intracranial aneurysms. J Neurosurg 1968;28:14–20
5. Jennett B, Bond M. Assessment of outcome after severe brain damage: a practical scale. Lancet 1975;1:480–484
6. Newell DW, Eskridge JM, Mayberg MR, et al. Angioplasty for the treatment of symptomatic vasospasm following subarachnoid hemorrhage. J Neurosurg 1989;71:654–660
7. Firlik KS, Kaufmann AM, Firlik AD, et al. Intra-arterial papaverine for the treatment of cerebral vasospasm following aneurysmal subarachnoid hemorrhage. Surg Neurol 1999;51: 66–74
8. Kassell NF, Helm G, Simmons N, et al. Treatment of cerebral vasospasm with intra-arterial papaverine. J Neurosurg 1992;77: 848–852
9. Clyde BL, Firlik AD, Kaufmann AM, et al. Paradoxical aggravation on vasospasm with papaverine infusion following aneurysmal subarachnoid hemorrhage: case report. J Neurosurg 1996;84: 690–695
10. McAuliffe W, Townsend M, Eskridge JM, et al. Intracranial pressure change induced during papaverine infusion for treatment of vasospasm. J Neurosurg 1995;83:430–434
11. Mathis JM, DeNardo A, Jensen ME, et al. Transient neurologic event associated with intra-arterial papaverine infusion for subarachnoid hemorrhage-induced vasospasm. AJNR Am J Neuroradiol 1994;15:1671–1674
12. Kallmes DF, Jensen ME, Dion JE. Infusing doubt into the efficacy of papaverine. AJNR Am J Neuroradiol 1997;18:263–264

62

CSF Drainage for Prevention and Reversal of Cerebral Vasospasm After Surgical Treatment of Intracranial Aneurysms

TALAT KIRIS, M.D., TÜLIN ERDEN, M.D., MÜGE SAHINBAS, M.D., BÜLENT OMAY, M.D., FIGEN ESEN, M.D.

Abstract

This study examined the effectiveness of cerebrospinal fluid (CSF) drainage used in addition to hemodynamic therapy and nimodipine to prevent cerebral vasospasm in patients operated on after aneurysmal subarachnoid hemorrhage (SAH). The study included 41 patients operated on for ruptured intracranial aneurysms between 2000 and 2003 in the Department of Neurosurgery of Istanbul University. All patients were operated by the senior author within 14 days of the hemorrhage. All patients received nimodipine (60 mg every 6 hours) and hemodynamic therapy in a neurological intensive care unit. CSF drainage was utilized in patients showing clinical signs of vasospasm or who had a high Fisher grade SAH on cranial computed tomography. Twenty (49%) patients were operated within 72 hours of SAH, and 21 (41%) were operated between day 3 and 11 post-SAH. Of 49 aneurysms, 48 were clipped in a single operative session. Clinical vasospasm was diagnosed in 18 patients (44%), and all were included in the group of 27 patients in whom CSF drainage procedures were conducted. Two patients had hydrocephalus on follow-up. No vasospasm-related permanent neurological deficit occurred. On discharge, 31 (76%) patients were in good condition according to the Glasgow outcome scale, four (10%) were moderately disabled, three (7%) were severely disabled, and three (7%) patients died. Unfavorable results were caused by direct effects of the hemorrhage or were related to surgical complications. This study suggests that CSF drainage is an effective treatment method for decreasing cerebral vasospasm in the postoperative period after aneurysmal SAH. CSF drainage removes vasospastic compounds from the subarachnoid space, decreases intracranial pressure, increases cerebral perfusion pressure, and decreases the incidence of hydrocephalus.

Delayed ischemic neurological deficits due to the cerebral vasospasm constitute a major complication of subarachnoid hemorrhage (SAH). Patients who develop vasospasm are more likely to die or be permanently disabled after SAH. The current therapy for cerebral vasospasm consists of the administration of

the Ca^{2+} antagonist nimodipine and hemodynamic (triple-H) therapy. Nimodipine is only partially effective in treating the consequences of vasospasm. Although it is widely used, the efficacy of triple-H therapy in preventing the onset of cerebral vasospasm and improving ischemic neurological deficits remains uncertain. A variety of drugs have been investigated experimentally and clinically for their ability to prevent or reverse the constriction of cerebral arteries after SAH, but none has gained general acceptance. In this study, we retrospectively evaluated our treatment protocol for cerebral vasospasm after the surgical treatment of ruptured intracranial aneurysms with a special emphasis on cerebrospinal fluid (CSF) drainage.

Materials and Methods

The study included 41 patients (21 male, 20 female) with aneurysmal SAH who were operated on at the Department of Neurosurgery, Istanbul Medical School, Istanbul University between January 2000 and February 2003. Their ages ranged from 13 to 80 with a mean age of 50 years. All patients underwent clipping of their aneurysms within 14 days of SAH by one surgeon (T.K.). The amount of subarachnoid blood on preoperative cranial computed tomography (CT) was classified according to the Fisher scale.[1] Preoperative neurological condition of the patients was evaluated with the World Federation of Neurological Surgeons scale.[2] After the operation all patients were admitted to the neurosurgical intensive care unit. Blood pressure (invasive), central venous pressure, body temperature, blood gases, and electrolytes were monitored. Neurological status was followed by frequent neurological examinations by intensive care nurses, neurosurgery and intensive care residents, and attendants. The patients were given nimodipine 60 mg orally every 4 hours. Hemodynamic therapy was induced by administration of crystalloid, colloid, and fresh frozen plasma. Noradrenalin was used to maximize the cardiac function. Hemodynamic therapy was estimated to be most effective if target values of systolic blood pressure of 150 to 180 mmHg, central venous pressure of 8 to 12 cm H_2O, and hematocrit of 28 to 30% were reached. CSF drainage once or twice a day via lumbar puncture was performed in patients who demonstrated symptoms of vasospasm and/or had a Fisher grade 3 to 4 SAH on a postoperative CT scan. External ventricular drainage catheters were inserted in patients with high neurological grades. The diagnosis of clinical vasospasm was made based on the following criteria: (1) the onset of major symptoms of vasospasm including insidious onset of confusion, disorientation, or decline in level of consciousness and focal deficit such as speech difficulty or new onset weakness; (2) the onset of minor symptoms of vasospasm including either or both worsening of headache and low grade fewer in an infection-free patient; (3) when no other identifiable cause of neurological deterioration existed, such as electrolyte disturbance, hypoxia, or seizures.[3]

Results

Twenty (49%) patients were operated on within 72 hours of SAH, 12 (29%) between 4 and 7 days, five (12%) between 8 and 11 days, and four (10%) between 12 and 14 days after SAH (Table 62–1).

TABLE 62–1 Clinical and Radiological Features and Outcome in 41 Patients with Subarachnoid Hemorrhage

Clinical Feature	Number of Patients
Sex	
Male	21 (51%)
Female	20 (49%)
WFNS grade	
1	22 (54%)
2	7 (17%)
3	8 (19%)
4	2 (5%)
5	2 (5%)
Fisher grade	
1	1 (2%)
2	18 (44%)
3	11 (27%)
4	11 (27%)
Day of surgery	
0–3	20 (49%)
4–7	12 (29%)
8–11	5 (12%)
12–14	4 (10%)
Location of aneurysm	
Middle cerebral artery	21 (43%)
Anterior communicating artery	13 (27%)
Posterior communicating artery	5 (10%)
Ophthalmic artery	4 (8%)
Anterior choroidal artery	3 (6%)
Internal carotid artery bifurcation	1 (2%)
Distal anterior cerebral artery	1 (2%)
Superior cerebellar artery	1 (2%)
Glasgow outcome scale	
Good recovery	31 (76%)
Moderate disability	4 (10%)
Severe disability	3 (7%)
Dead	3 (7%)

WFNS, World Federation of Neurological Surgeons.

Neurological grades on the World Federation scale were grade 1 in 22 patients (54%), grade 2 in seven patients (17%), grade 3 in eight patients (19%), grade 4 in two patients (5%), and grade 5 in two patients (5%). Eight patients harbored multiple aneurysms. There were 49 aneurysms in total, and 48 were clipped at the initial craniotomies.

CSF drainage was performed in 21 patients. In nine patients the indication for CSF drainage was a postoperative CT scan showing Fisher grade 3 or 4 SAH. Six of these patients (66%) developed vasospasm. Eighteen (44%) patients, including those six patients previously mentioned, showed symptoms of clinical vasospasm. Major symptoms accounted for 11 (27%) and minor symptoms for 10 (24%) of the patients. The method of CSF drainage was external ventricular drainage in six patients and intermittent lumbar punctures in 16 patients. Both methods were used in one patient. Lumbar punctures were performed once a day in seven patients and twice a day in nine. Fifteen to 20 mL CSF was removed at each lumbar puncture.

Table 62–2 shows the relationship between vasospasm and Fisher grade and day of surgery. Two patients among three with vasospasm who were operated between days 12 to 14 already had developed vasospasm when they were taken to surgery. Two patients developed hydrocephalus on follow-up and were treated by ventriculoperitoneal shunt placement. Bacterial meningitis occurred in four patients, and this was treated with antibiotics. On discharge 31 (76%) patients were in good condition according to the Glasgow outcome scale. Four (10%) were moderately disabled, three (7%) were severely disabled, and three (7%) patients died.[4] There were no permanent neurological deficits secondary to vasospasm. Unfavorable results were thought to be caused by either a direct effect of the hemorrhage or to surgical complications.

TABLE 62–2 Relationship between Vasospasm and Timing of Surgery and Fisher Grades

Subarachnoid Hemorrhage—Operation (days)	Vasospasm
0–3	8/20 (40%)
4–7	5/12 (42%)
8–11	2/5 (40%)
12–14	3/4 (75%)
Fisher grade	Vasospasm
1	0/1 (0%)
2	4/18 (22%)
3	6/11 (55%)
4	8/11 (73%)

Discussion

Outcome in patients with aneurysmal SAH has improved over the past 20 years. Early aneurysm clipping prevents rebleeding but cerebral ischemia due to vasospasm remains an important cause of mortality and morbidity. Several previous studies have demonstrated that high concentrations of vasoactive compounds acting as potent spasmogenic metabolites can be identified in the CSF after SAH.[5–8] Hemoglobin and breakdown products generated from or by it, including oxyhemoglobin, are thought to be key spasmogens.[9,10] The metabolites of hemoglobin rapidly bind and destroy nitric oxide (NO) and prevent its entry into vascular smooth muscle cells. This disrupts the balance of dilating and constricting factors and results in vasoconstriction.[11] These metabolites may have additional direct effects on NO synthase-containing nerve fibers in the adventitia of the cerebral arteries, and they can also penetrate into the arterial wall and inhibit endothelial NO synthase. These effects are responsible for prolonged constriction of conductive cerebral vessels after SAH.[12]

It is at least theoretically plausible that removal of CSF from patients with SAH could reduce the amount of breakdown products of hemoglobin and prevent or reverse cerebral vasospasm. In a series of 185 patients operated on for aneurysmal SAH, the incidence of vasospasm was 11% in 150 patients with CSF drainage and 29% in 35 patients without drainage.[13] Ito et al presented data from 25 patients with SAH who underwent aneurysmal neck clipping and cisternal CSF drainage. The effect of drainage was graded as fair (150 mL/day), moderate (50–149 mL/day), or poor (< 49 mL/day). Symptomatic vasospasm occurred in 78, 60, and 42% of patients with poor, moderate, and fair drainage, respectively.[14] Contrary to this report was the study of Kasuya and coworkers.[15] Ninety-two patients were operated on for ruptured aneurysms within 48 hours of SAH. Patients with higher drainage volumes in the early period of SAH had a higher incidence of cerebral infarction later on.[15]

In the present study, CSF drainage was used in patients with Fisher grade 3 and 4 SAH on postoperative CT scans. The purpose was to drain spasmogenic blood and thereby reduce the risk of vasospasm. We noted that 66% of patients treated this way developed clinical vasospasm. In 18 cases with symptoms of vasospasm, reversal of symptoms was possible with CSF drainage and hemodynamic therapy. In 16 patients, CSF drainage was achieved by repeated lumbar punctures. Removal of a large amount of CSF with continuous drainage

could collapse the subarachnoid space and prevent its irrigation, thus retaining spasmogens in the vicinity of the arteries. Good results have been obtained by the intermittent drainage method developed by Auer and Mokry, who realized that this procedure forced CSF through its normal pathways during the intervals between drainage, theoretically allowing restoration of normal CSF circulation.[16] It has been demonstrated that SAH is associated with high intracranial pressure and low cerebral perfusion pressure. A study by Fukuhara et al noted that patients with high intracranial pressure tended to have more severe, prolonged, and diffuse vasospasm compared with patients with low intracranial pressure.[17] Finally, Lang and colleagues demonstrated that cerebral autoregulation impairment precedes vasospasm and ongoing vasospasm worsens autoregulation, resulting in low cerebral perfusion pressure.[18] Thus removal of CSF would also be beneficial in reducing intracranial pressure, increasing cerebral perfusion pressure, and augmenting cerebral blood flow.

Conclusion

This study demonstrates that CSF drainage is an effective method for reversing the symptoms of clinical vasospasm related to aneurysmal SAH.

REFERENCES

1. Fisher CM, Kistler JP, Davis JM. Relation of cerebral vasospasm to subarachnoid hemorrhage visualized by computerized tomographic scanning. Neurosurgery 1980;6:1–9
2. Drake CG, Hunt WE, Sano K, et al. Report of World Federation of Neurological Surgeons Committee on a Universal Subarachnoid Hemorrhage Grading Scale. J Neurosurg 1988;68:985–986
3. Haley EC Jr, Kassell NF, Torner JC. A randomized controlled trial of high-dose intravenous nicardipine in aneurysmal subarachnoid hemorrhage: a report of the Cooperative Aneurysm Study. J Neurosurg 1993;78:537–547
4. Jennett B, Bond M. Assessment of outcome after severe brain damage: a practical scale. Lancet 1975;1:480–484
5. Suzuki K, Meguro K, Sakurai T, Saitoh Y, Takeuchi S, Nose T. Endothelin-1 concentrations increase in the cerebrospinal fluid in cerebral vasospasm caused by subarachnoid hemorrhage. Surg Neurol 2000;53:131–135
6. Seifert V, Löffler BM, Zimmermann M, Roux S, Stolke D. Endothelin concentrations in patients with aneurysmal subarachnoid hemorrhage. J Neurosurg 1995;82:55–62
7. Chehrazi BB, Giri S, Joy RM. Prostaglandins and vasoactive amines in cerebral vasospasm after aneurysmal subarachnoid hemorrhage. Stroke 1989;20:217–224
8. Macdonald RL, Weir BK, Runzer TD, Grace MG. Malondialdehyde, glutathione peroxidase, and superoxide dismutase in cerebrospinal fluid during cerebral vasospasm in monkeys. Can J Neurol Sci 1992;19:326–332
9. Macdonald RL, Weir BKA. A review of hemoglobin and the pathogenesis of cerebral vasospasm. Stroke 1991;22:971–982
10. Asano T. Oxyhemoglobin as the principal cause of cerebral vasospasm: a holistic view of its actions. Crit Rev Neurosurg 1999;9:303–318
11. Afshar JKB, Pluta RM, Boock RJ, Thompson BG, Oldfield EH. Effect of intracarotid nitric oxide on primate cerebral vasospasm after subarachnoid hemorrhage. J Neurosurg 1995;83: 118–122
12. Pluta RM, Thompson BG, Afshar JK, Boock RJ, Juliano B, Oldfield EH. Nitric oxide and vasospasm. Acta Neurochir Suppl 2001;77: 67–72
13. Sonobe M, Takahashi S, Koshu K, Hiroto S. Prevention of intracranial arterial vasospasm using combined ventriculocisternal shunting and cisternal drainage. In: Wilkins RH, ed. Cerebral Vasospasm. New York: Raven Press; 1988: 409–414
14. Ito U, Tomita H, Yamazaki S, Takada Y, Inaba Y. Enhanced cisternal drainage and cerebral vasospasm in early aneurysm surgery Acta Neurochir (Wien) 1986;80:18–23
15. Kasuya H, Shimizu T, Kagawa M. The effect of continuous drainage of cerebrospinal fluid in patients with subarachnoid hemorrhage: a retrospective analysis of 108 patients. Neurosurgery 1991;28:56–59
16. Auer LM, Mokry M. Acute aneurysm surgery, disturbed CSF circulation, intracranial pressure and hydrocephalus. In: Auer LM, ed. Timing of Aneurysm Surgery. Berlin and New York: Walter de Gruyer; 1985:331–341
17. Fukuhara T, Douville CM, Eliott JP, Newell DW, Winn HR. Relationship between intracranial pressure and the development of vasospasm after aneurysmal subarachnoid hemorrhage. Neurol Med Chir (Tokyo) 1998;38:710–715
18. Lang EW, Diehl RR, Mehdorn HM. Cerebral autoregulation testing after aneurysmal subarachnoid hemorrhage: the phase relationship between arterial blood pressure and cerebral blood flow velocity. Crit Care Med 2001;29:158–163

63

Cerebral Vasospasm Is Markedly Reduced by Lumbar Cerebrospinal Fluid Drainage

RICHARD H. SCHMIDT, M.D., PH.D. AND
PAUL KLIMO JR., M.D., M.PH.

Abstract

Vasospasm after subarachnoid hemorrhage (SAH) is related to the quantity of hemorrhage in the subarachnoid cisterns. This has led us to postulate that cerebrospinal fluid (CSF) drainage via the lumbar cistern will reduce vasospasm by accelerating the clearance of subarachnoid blood and related spasmogens. We have analyzed the incidence of vasospasm in all patients with SAH managed at this institution since October 1993 (n = 250). We excluded from analysis patients who were in poor neurological condition on admission or following aneurysm treatment (n = 36), those with a delay from SAH to admission of 4 or more days (n = 13), and patients with Fisher grade 1 or 2 hemorrhage (n = 40). All patients were managed with a consistent hemodynamic therapy protocol, nimodipine, and transcranial Doppler sonography. Patients were managed with (n = 81) or without (n = 80) lumbar CSF drainage postoperatively throughout the risk period for vasospasm. Allocation to these groups was based on the practice of the attending neurosurgeon. The two groups were equivalent with respect to age, clinical grade, and aneurysm location. Patients in the lumbar drainage group who required initial ventricular drains for hydrocephalus were converted to lumbar drainage postoperatively. The lumbar drain group had significantly ($p < .0001$ to $p < .01$) less cerebral vasospasm as determined by three different assessments: presence of clinical ischemic deficit (17% vs 53%), use of angioplasty (17% vs 49%), and presence of infarction secondary to vasospasm (7% vs 30%). Neurological outcome at the time of discharge was significantly better in the lumbar drainage group as well (55% of patients discharged home vs 25%). Good recovery was deemed to have occurred at 1 to 3 months in 74% of those treated with lumbar drainage compared with only 35% of those without. Hospital length of stay was decreased from 21 days to 17 days. A similar degree of benefit was seen in the subgroup of patients who presented with acute hydrocephalus and had ventricular drains placed initially. There was no permanent morbidity attributed to use of lumbar drainage. Within the limits of a retrospective study, these data suggest that lumbar CSF drainage reduces vasospasm in patients with dense SAH. A prospective trial of this intervention is now being started.

Current medical and endovascular therapy for vasospasm does not prevent its occurrence but can mitigate its effects. Abundant research has identified breakdown products of blood in the subarachnoid spaces as being the primary inducer of vasospasm.[1] We have hypothesized that lumbar cerebrospinal fluid (CSF) drainage during the postoperative period may reduce the occurrence of vasospasm by promoting CSF circulation through the subarachnoid spaces and thereby facilitating the clearance of blood.

Patients and Methods

At our institution over the past 9 years, half of the neurosurgeons who have treated patients with aneurysms have preferentially managed aneurysmal subarachnoid hemorrhage (SAH) patients with a protocol incorporating lumbar CSF drainage. Lumbar CSF drainage is initiated only after a postoperative computed tomographic (CT) scan shows no contraindication such as ventricular obstruction, hematoma, or other lesion causing shift or placing the patient at risk of downward herniation. The goal of CSF drainage is to drain 5 to 10 mL/hour by adjusting drain height to 5 to 15 cm above the external auditory canal. Drainage is continued until the CSF is no longer visibly hemorrhagic and the patient is beyond the risk period for vasospasm. We have examined the effect of this protocol on the occurrence of clinically symptomatic vasospasm, the need for endovascular rescue therapies, the occurrence of vasospasm-related cerebral infarction, and patient outcome. The control group for this study consisted of those patients managed with a conventional protocol of either external ventricular CSF drainage or no drain. The call schedule served to pseudorandomize patient assignment to either of these methods.

Apart from the method of CSF drainage, all of these patients were managed similarly. Patients with symptomatic acute hydrocephalus or elevated intracranial pressure on admission were treated with ventricular drainage. Study patients were subsequently converted to lumbar drainage in the postoperative period. All patients underwent early (within 24–36 hours) surgical clipping, nimodipine, hemodynamic therapy guided by central venous catheters, daily transcranial Doppler examinations, and close monitoring in a neurosurgical intensive care unit. Patients with neurological deficits from vasospasm that were refractory to induced hypertension were treated with either balloon angioplasty or repeated intra-arterial infusion of papaverine.

For the purpose of this study, we selected only patients who were in good neurological condition following initial resuscitation and aneurysm treatment, and those at high risk of developing vasospasm (Fisher grade 3 or higher hemorrhages).[2] Patients with both dense subarachnoid blood and intraparenchymal or intraventricular hemorrhage of > 5 mL were designated as being Fisher grade 3 + 4. Outcome was assessed at 1 to 3 months post-SAH by the Glasgow outcome score.[3]

Results

Of 250 patients with aneurysmal SAH treated during the period of study, we excluded from analysis patients with Fisher grade 1 and 2 SAH (n = 40), 14 patients admitted more than 4 days after SAH, 24 Hunt and Hess grade 5[4] patients who failed to improve with treatment, and 12 patients with severe neurological deficits following surgery. There were 81 patients in the final lumbar drain treatment cohort and 80 patients in the control cohort. The two groups of patients were well matched with respect to age, sex, aneurysm types, and Hunt and Hess scores. The control cohort contained a higher percent of patients with Fisher 3 + 4 hemorrhages and patients needing initial external ventricular drainage due to our precautions against using lumbar drains in patients with significant intracerebral hemorrhages or ventricular obstruction. Subgroup analysis and multivariate regression analysis was performed to account for this imbalance.

The results of lumbar CSF drainage on vasospasm for the entire study group are depicted in **Figure 63–1**. Clinically symptomatic vasospasm (delayed ischemic deficit), need for endovascular therapy for vasospasm, and cerebral infarction attributed to vasospasm were dramatically reduced in the cohort with lumbar CSF drainage. For each of these end

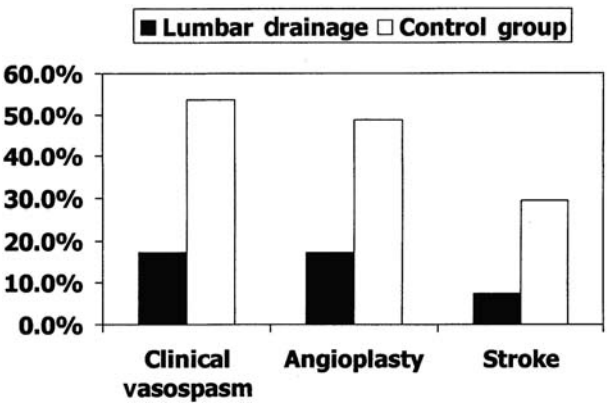


FIGURE 63–1 Percent of patients with each of the three markers of vasospasm who had lumbar drainage. Lumbar drainage reduced the relative risk of clinically symptomatic vasospasm, need for endovascular treatment, and vasospasm-related cerebral infarction by 68, 65, and 75%, respectively ($p \leq .001$).

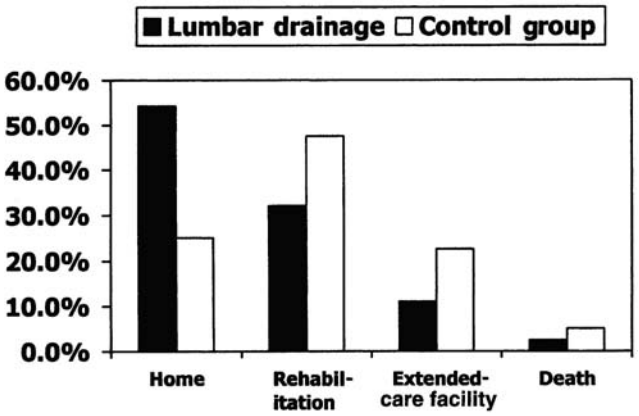


FIGURE 63–2 Patient disposition at time of discharge for patients with lumbar drainage and the control group that did not have lumbar drainage. More patients with lumbar drainage were discharged to home than patients without lumbar drainage.

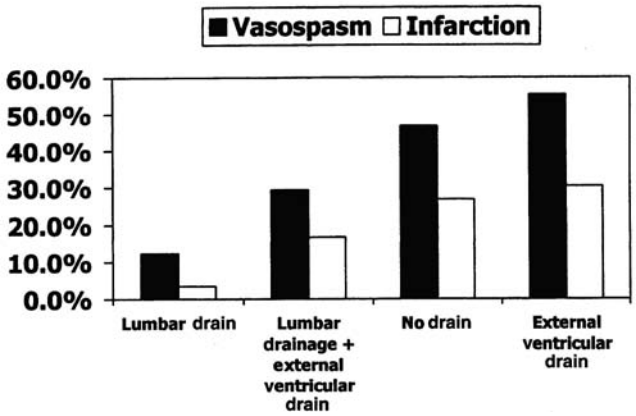


FIGURE 63–3 Effect of different cerebrospinal fluid drainage techniques on the incidence of clinically symptomatic vasospasm and vasospasm-induced cerebral infarction according to whether patients had only lumbar drainage, lumbar drainage after external ventricular drainage, no drainage procedure, or external ventricular drainage only.

points, the relative risk reduction was between 65 and 75% ($p \leq .001$ for all measures). Fifty-four percent of patients in the lumbar drain group were discharged directly home compared with 26% in the control cohort (Fig. 63–2). There were correspondingly fewer patients needing rehabilitation or convalescent care and fewer deaths among those treated with lumbar drainage. At 1 to 3 months follow-up, 70% of the lumbar drain cohort had a Glasgow outcome score of 5 (good recovery) compared with 35% in the control group. Hospital length of stay was reduced from 21 days for the controls to 17 days in the lumbar drain group ($p \leq .0006$).

Subgroup analyses were performed within individual Fisher grades and in the group of patients who had placement of ventricular drains on admission. In each of these subgroups, lumbar CSF drainage continued to show benefits over conventional treatment. In the Fisher grade 3 group, lumbar CSF drainage resulted in a relative risk reduction of 70, 61, and 82% in symptomatic vasospasm, need for endovascular therapy for vasospasm, and cerebral infarction attributed to vasospasm ($p \leq .001$). In the Fisher 3 + 4 group, the relative risk reductions were 51, 51, and 52%, respectively. In those patients treated initially with ventricular drainage, relative risk reductions with subsequent lumbar CSF drainage were 71, 69, and 66%. Multivariate logistic regression analysis found that for each of our vasospasm outcome measures, the strongest predictive factors related to vasospasm were, in decreasing order, presence of lumbar CSF drainage ($p \leq .001$), Fisher grade, and Hunt and Hess grade. Nonsignificant factors included age, intraoperative aneurysm rupture, and placement of a ventricular drain on admission.

Figure 63–3 depicts the occurrence of symptomatic vasospasm and cerebral infarction in each of the various drain subgroups. The lowest incidence of these problems occurred in patients who underwent only lumbar CSF drainage, followed by the cohort who had ventricular drainage and were converted to lumbar drainage. Patients with no form of CSF drainage had the third highest incidence of vasospasm and infarction, whereas the highest incidences were found in the group with only ventricular drainage.

Need for shunt placement was somewhat lower in the lumbar drain cohort (24% vs 36%), but this did not reach statistical significance. Drain complications were similar in both groups with an equal number developing catheter breakage and infection (two of each condition in each drain group). Two patients who had external ventricular drains developed catheter-related hemorrhages requiring surgical evacuation. In the lumbar drain group three patients developed transient signs of neurological worsening when the drains were first opened. In all cases this cleared with closure of the drains, and these patients all tolerated drain reopening within the next 24 hours, but at a decreased rate of drainage. No lumbar drain patient developed any permanent adverse effects that could be attributed to the drain.

Discussion

Within the limits of a nonrandomized retrospective study, our data strongly suggest that postoperative lumbar CSF drainage is effective at decreasing cerebral vasospasm, improving clinical outcome, and decreasing hospital length of stay. Although not specifically quantified in this study, observation of daily transcranial

Doppler flow velocities and postoperative angiograms suggested that lumbar drainage appears to prevent vasospasm rather than promote cerebral perfusion in the face of arterial narrowing. This is consistent with the concept of CSF-soluble spasmogenic agents released by the lysis of subarachnoid hematoma. We believe that lumbar drainage works by promoting the laminar flow of clean CSF from its production site in the ventricles, into and through the subarachnoid cisterns, and out the lumbar theca, facilitating the clearance of blood and spasmogens. Conversely, drainage directly from the ventricle may promote cisternal CSF stasis. Support for this hypothesis was our observation that patients with ventricular drainage had the highest risk of vasospasm.

The strategy of lumbar CSF drainage is similar to approaches that rely on the use of thrombolytics in that both interventions strive to clear blood from the basal cisterns. Findlay et al noted a trend for decreased vasospasm in a prospective trial of a single dose of tissue plasminogen activator instilled at the time of surgery.[5] Kodama and colleagues reported an uncontrolled case series of 217 patients who were treated with a combination of cisternal drainage and continuous intrathecal irrigation with urokinase and ascorbic acid.[6] The incidence of vasospasm in these patients was among the lowest ever reported in the literature. Thus, interventions that promote the removal of cisternal blood appear to prevent vasospasm. Lumbar drainage may be safer and more easily performed than cisternal irrigation. Strict attention to appropriate precautions is necessary, however, when using lumbar drainage in postoperative patients. A randomized prospective trial of this treatment is currently under way to confirm these initial findings.

REFERENCES

1. Macdonald RL. Pathophysiology and molecular genetics of vasospasm. Acta Neurochir Suppl 2001;77:7–11
2. Fisher CM, Kistler JP, Davis JM. Relation of cerebral vasospasm to subarachnoid hemorrhage visualized by computerized tomographic scanning. Neurosurgery 1980;6:1–9
3. Jennett B, Bond M. Assessment of outcome after severe brain damage: a practical scale. Lancet 1975;1:480–484
4. Hunt WE, Hess RM. Surgical risk as related to time of intervention in the repair of intracranial aneurysms. J Neurosurg 1968;28:14–20
5. Findlay J, Kassell N, Weir B, et al. A randomized trial of intraoperative, intracisternal tissue plasminogen activator for the prevention of vasospasm. Neurosurgery 1995;37:168–178
6. Kodama N, Sasaki T, Kawakami M, et al. Cisternal irrigation therapy with urokinase and ascorbic acid for prevention of vasospasm after aneurysmal subarachnoid hemorrhage: outcome in 217 patients. Surg Neurol 2000;53:110–117

64

Fenestration of the Lamina Terminalis Reduces Vasospasm After Subarachnoid Hemorrhage from Anterior Communicating Artery Aneurysms

NORBERTO ANDALUZ, M.D. AND MARIO ZUCCARELLO, M.D.

Abstract

Vasospasm after subarachnoid hemorrhage (SAH) relates to the amount of subarachnoid blood. We hypothesized, therefore, that fenestration of the lamina terminalis would reduce the incidence of vasospasm in patients with SAH from anterior communicating artery aneurysms by promoting the clearance of the spasmogenic blood. A prospective study was performed on 65 women and 35 men (mean age 55 years, range 18–84 years) with Fisher grade 3 SAH who underwent clipping or coiling of anterior communicating artery aneurysms before day 3 post-SAH. Of these, 49 underwent fenestration of the lamina terminalis and 51 did not (43 clipped, eight coiled). We evaluated admission and discharge clinical grades, clinical vasospasm, interventional vasospasm therapy, and permanent shunting. Follow-up ranged from 7 to 35 months. Hunt and Hess scores were grade 1 in eight patients, 2 in 41,3 in 34,4 in 13, and 5 in four. Clinical vasospasm affected 27 (53%) patients without and 16 (33%) with fenestration of the lamina terminalis ($p < 0.01$). Interventional therapy for vasospasm was required in 18 (66%) and eight (50%) patients, respectively. In the group without fenestration, 16 (31%) deaths occurred of which nine were related to vasospasm. In the group undergoing fenestration, six (12%) deaths occurred, with four related to vasospasm. At discharge, Glasgow outcome scores were good (good recovery or moderate disability) in 33 (67%) with fenestration and 18 (35%) without fenestration. At latest follow-up, five (15%) without fenestration and two (5%) with fenestration of the lamina terminalis required permanent shunting ($p < .01$). Fenestration of the lamina terminalis was associated with a decreased incidence of cerebral vasospasm and with better outcome. Poor outcomes were associated with higher Hunt and Hess scores, need for ventricular drainage, elevated intracranial pressure, and multiple interventional procedures for vasospasm. No complications were linked to fenestration of the lamina terminalis.

The clinical course of aneurysmal subarachnoid hemorrhage (SAH) is plagued by medical and neurosurgical complications that negatively affect outcome. Of the latter, most of the morbidity and mortality is claimed by hydrocephalus and vasospasm. A handful of reports and anecdotal data have been published suggesting that fenestration of the lamina terminalis during aneurysm surgery in patients with SAH may

improve outcome and may decrease the rate of ventriculoperitoneal shunting for post-SAH hydrocephalus.[1–3] These reports led us to evaluate prospectively the effects of fenestration of the lamina terminalis in a population of patients who were judged to be at high risk for the development of post-SAH complications.

Studies have documented that the rates of vasospasm and post-SAH hydrocephalus relate to the amount of blood in the subarachnoid spaces. Therefore, patients with a Fisher grade 3 SAH on cranial computed tomography (CT) constitute a group at particular risk.[4] Anterior communicating artery aneurysms are associated with the highest incidence of hydrocephalus and the worst outcomes of anterior circulation aneurysms. Finally, the lamina terminalis is intimately related to the anterior communicating artery complex and is always exposed during surgical approach to this area. All these considerations make Fisher grade 3 SAH patients with anterior communicating artery aneurysms particularly susceptible for the development of complications and poor outcomes. Thus these select patients may benefit from a simple procedure like fenestration of the lamina terminalis, which adds no morbidity and requires minimal operative time.

Materials and Methods

From 2000 to 2003, 100 Fisher grade 3 patients (65 women and 35 men; mean age 55 years, range 18–84 years) underwent clipping or coiling of anterior communicating artery aneurysms before post-SAH day 3. Forty-nine underwent fenestration of the lamina terminalis and 51 did not, including 43 who underwent clipping and eight who underwent coiling. Patient allocation to either group was based on the surgeon's preference. We prospectively evaluated admission and discharge clinical grades, presence of hydrocephalus at admission, treatment modality, occurrence of clinical vasospasm, need for interventional vasospasm therapy, and need for permanent ventriculoperitoneal shunting. Follow-up ranged 7 to 35 months.

After surgery or coiling, standard medical management was used, including administration of nimodipine, volume expansion, and maintenance of optimal general hemodynamic and medical status. Transcranial Doppler measurements were performed daily in all patients from SAH days 3 through 14. Interventional neuroradiological procedures were performed based on our treatment algorithm for vasospasm.[5] For all patients undergoing microsurgical aneurysm clipping, meticulous cisternal toilette of the subarachnoid clot was performed to the maximum extent. In those subjected to fenestration of the lamina terminalis, a longitudinal incision along the avascular midline of the lamina terminalis was performed with a No. 11 scalpel blade or microscissors after precise identification of the surrounding neurovascular structures. Hydrocephalus was diagnosed when the ventricular size exceeded the 95th percentile in the cerebroventricular index adjusted for patient age. The decision to proceed with ventriculoperitoneal shunting was based on clinical and radiological findings.

Results

Of 100 patients included in this analysis, 49 underwent lamina terminalis fenestration and 51 did not. There were no statistically significant differences between groups regarding admission Hunt and Hess scores[6] and age. In the group that did not undergo fenestration, 43 patients were treated surgically and eight had endovascular treatment with Guglielmi detachable coils. All patients in the fenestration group were surgically treated. Clinical vasospasm affected 27 (53%) patients in the nonfenestration group and 16 (31%) in the fenestration group ($p < .001$). Interventional therapy for vasospasm was required in 18 (66%) patients without lamina terminalis fenestration and 8 (50%) with fenestration (not statistically significant). Sixteen (31%) patients who did not have fenestration and 6 (12%) who did have fenestration died ($p < .001$, Fig. 64–1). Clinical vasospasm was related to these deaths in nine and four patients, respectively. At discharge, Glasgow outcome scores were good (good recovery or moderate disability) in 33 (67%) of patients undergoing and 18 (35%) not undergoing fenestration of the lamina terminalis ($p < .001$, Fig. 64–2). Poor outcomes were associated with higher Hunt and Hess scores, hydrocephalus on admission, need for ventricular drainage (regardless of duration), elevated intracranial pressure, clinical vasospasm, and need for interventional therapy for vasospasm. At latest follow-up for patients surviving SAH, ventriculoperitoneal shunting was performed in five (15%) of 34 patients who did not have and only two (5%) of 43 who did have fenestration of the lamina terminalis ($p < .001$). No complications were linked to fenestration of the lamina terminalis.

Discussion

The fairly well accepted and likely factual observation that the incidence of vasospasm in SAH patients relates to the amount of blood in the subarachnoid spaces has led some physicians to propose that cisternal drainage may decrease the incidence of vasospasm. On the other hand, others have clearly demonstrated the coexistence of hydrocephalus and cerebral vasospasm in more than 60% of SAH patients.[7,8]

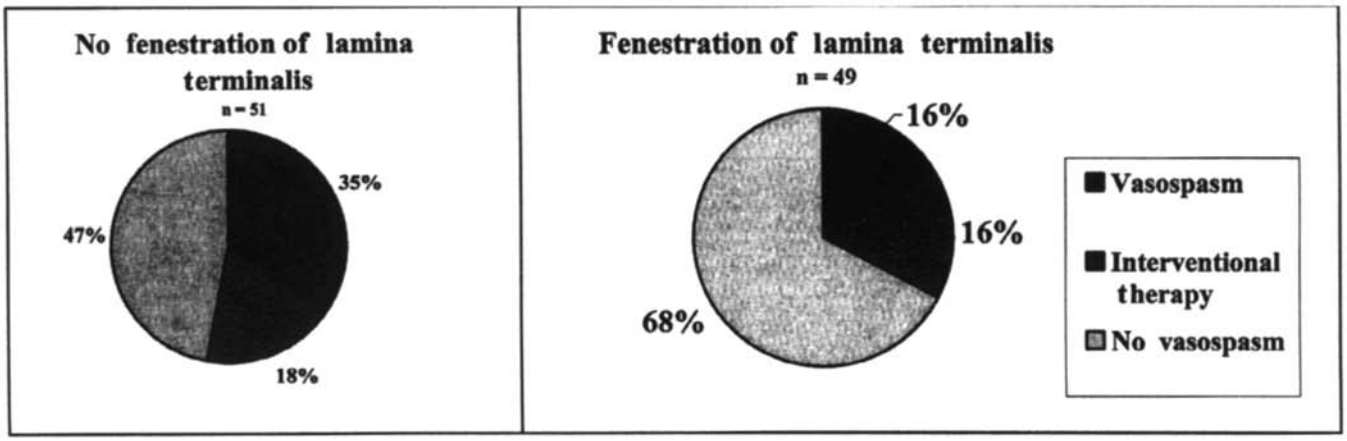


FIGURE 64–1 The incidence of cerebral vasospasm in patients with and without fenestration of the lamina terminalis. Patients are classified by whether or not they developed vasospasm. Patients undergoing interventional therapy for vasospasm are also indicated. (Printed with permission by Mayfield Clinic.)

Decreased rates of cerebral vasospasm and improved outcomes were reported by Japanese and European authors with the use of either open cisternal drainage or fenestration of the lamina terminalis with or without the additional fenestration of the membrane of Liliequist.[1–3, 9–11] With meticulous surgical toilette of the basal cisterns, fenestration of the lamina terminalis, and insertion of a continuous cisternal lavage system with or without fibrinolytic agents (chiefly urokinase), several Japanese authors substantially reduced the rates of symptomatic and angiographic vasospasm.[9–11] The proposed mechanism is a reduction in the concentration of blood-derived spasmogenic agents in the cerebrospinal fluid in contact with the basal brain arteries. It has been calculated that the use of these techniques can lead to clearance of blood volumes of up to 100 mL/day and hemoglobin of up to 1 g/day. Furthermore, a drainage volume-dependent effect has been demonstrated. The shortcomings of this approach include the significant logistic effort, the very high incidence of ventriculoperitoneal shunting (up to 48% in some series),[9] and an infection rate of up to 9%.[10] Sindou proposed performing a third ventriculostomy through the lamina terminalis to establish an internal cisternal drainage mechanism.[2] This mechanism would then bypass obstructions of the ventricular system and provide a continuous irrigation mechanism generated by the cerebrospinal fluid pulse pressure, consequently improving cerebrospinal fluid dynamics and reducing concentrations of blood-derived spasmogenic substances. Using this approach, Sindou[2] and Tomasello et al[3] reduced shunting rates and improved outcomes compared with a control population. This effect was enhanced by the addition of the fenestration of the membrane of Liliequist.[2]

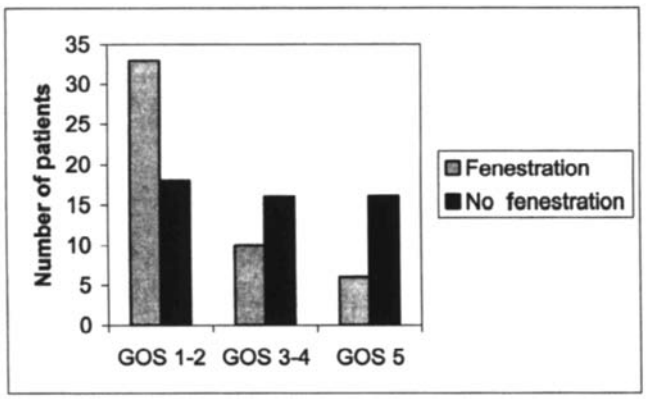


FIGURE 64–2 Distribution of Glasgow outcome scores (GOS 1–2: good recovery and moderate disability, GOS 3–4: severe disability and vegetative state, and GOS 5: dead) at discharge in patients with and without fenestration of the lamina terminalis. (Printed with permission by Mayfield Clinic.)

In our series, vasospasm developed in 53% of patients who did not undergo lamina terminalis fenestration and 33% who had fenestration ($p < .001$). A decreased need for multiple interventional neuroradiology sessions for clinical vasospasm was noted in patients undergoing fenestration of the lamina terminalis. Better outcomes were also statistically more likely compared with the nonfenestration group. Potential benefits of fenestration of the lamina terminalis including reduction of the incidence of vasospasm, although speculative, render enough promise to continue further clinical studies.

Conclusion

Fenestration of the lamina terminalis significantly decreased the incidence of post-SAH hydrocephalus and was associated with better outcomes in our patient population. The role of fenestration of the lamina terminalis

in the prevention of vasospasm appears less clear, although worthy of consideration in controlled studies. This safe and easy to perform maneuver is recommended during the surgical treatment of anterior communicating artery aneurysms, during which the lamina terminalis is readily exposed. For other aneurysms of the anterior circulation that require further dissection, the potential risks of fenestrating the lamina terminalis should be weighed against the benefits suggested here.

REFERENCES

1. Komotar RJ, Olivi A, Rigamonti D, Tamargo RJ. Microsurgical fenestration of the lamina terminalis reduces the incidence of shunt-dependent hydrocephalus after aneurysmal subarachnoid hemorrhage. Neurosurgery 2002;51:1403–1412
2. Sindou M. Favourable influence of opening the lamina terminalis and Lilliequist's membrane on the outcome of ruptured intracranial aneurysms: a study of 197 consecutive cases. Acta Neurochir (Wien) 1994;127:15–16
3. Tomasello F, d'Avella D, de Divitiis O. Does lamina terminalis fenestration reduce the incidence of chronic hydrocephalus after subarachnoid hemorrhage? Neurosurgery 1999;45:827–831
4. Fisher CM, Kistler JP, Davis JM. Relation of cerebral vasospasm to subarachnoid hemorrhage visualized by computerized tomographic scanning. Neurosurgery 1980;6:1–9
5. Andaluz N, Tomsick TA, Tew JM Jr, van Loveren HR, Yeh HS, Zuccarello M. Indications for endovascular therapy for refractory vasospasm after aneurysmal subarachnoid hemorrhage: experience at the University of Cincinnati. Surg Neurol 2002; 58:131–138
6. Hunt WE, Hess RM. Surgical risk as related to time of intervention in the repair of intracranial aneurysms. J Neurosurg 1968;28:14–20
7. Black PMcL. Hydrocephalus and vasospasm after subarachnoid hemorrhage from ruptured intracranial aneurysms. Neurosurgery 1986;18:12–16
8. Kolluri VR, Sengupta RP. Symptomatic hydrocephalus following aneurysmal subarachnoid hemorrhage. Surg Neurol 1984; 21:402–404
9. Inagawa T, Kamiya K, Matsuda Y. Effect of continuous cisternal drainage on cerebral vasospasm. Acta Neurochir (Wien) 1991; 112:28–36
10. Sakaki S, Ohta S, Kuwabara H, Shiraishi M. The role of ventricular and cisternal drainage in the early operation for ruptured intracranial aneurysms. Acta Neurochir (Wien) 1987;88:87–94
11. Kodama N, Matsumoto M, Sasaki T, Konno Y, Sato T. Cisternal irrigation therapy with urokinase and ascorbic acid for prevention of vasospasm. Acta Neurochir Suppl 2001;77:171–174

SECTION X

Clinical—Treatment

65

Clinical Trial of Nicardipine Prolonged-Release Implants for Prevention of Vasospasm

HIDETOSHI KASUYA, M.D., HIDEAKI ONDA, M.D., ATSUSHI SASAHARA, M.D., TOSHIYUKI SASAKI, M.D., HIROYUKI AKAGAWA, M.D., TAKU YONEYAMA, M.D., TOMOKATSU HORI, M.D.

Abstract

Despite extensive investigative efforts, there are few treatments that can prevent vasospasm following subarachnoid hemorrhage (SAH). This study examined the efficacy and safety of nicardipine prolonged-release implants for prevention of vasospasm in humans. This treatment was demonstrated to significantly reduce vasospasm in canine clot-placement and double-hemorrhage models. Fifty consecutive SAH patients with thick subarachnoid clot were treated with nicardipine implants (diameter 2 mm, length 10 mm, containing 4 mg of nicardipine) during surgery after clipping of their aneurysm between October 1999 and December 2002. The number and location of pellets depended on the amount and site of subarachnoid clot on preoperative computed tomography and on craniotomy. Two to 12 implants were placed in the cisterns of the internal carotid, middle cerebral, and/or anterior cerebral arteries where thick clots existed and therefore vasospasm related to delayed ischemic neurological deficits was highly likely to occur. Delayed ischemic neurological deficits and cerebral infarctions were seen in two patients. Angiography performed on seven to 12 days after SAH revealed no vasospasm in arteries adjacent to nicardipine implants. No complications were experienced. Vasospasm was completely prevented in arteries encased in thick clot when nicardipine implants were placed adjacent to the arteries during surgery. This drug-delivery system offers a promising approach for preventing vasospasm.

Vasospasm continues to be one of the primary causes of mortality and neurological morbidity in patients with aneurysmal subarachnoid hemorrhage (SAH). Despite extensive investigative efforts, there are few treatments that can prevent vasospasm in these patients. We have developed a drug-delivery system using a vasodilating drug (nicardipine) that can be implanted intracranially at the time of surgery for aneurysm clipping. There are no systemic or local side effects associated with long-term intrathecal drug administration through indwelling catheters.[1–4] We report herein the results of the first 50 consecutive patients treated with intrathecal nicardipine prolonged-release implants.[1]

Patients and Methods

Development of Nicardipine Prolonged-Release Implants for Humans

A rod-shaped pellet (2 mm in diameter, 10 mm in length, containing 4 mg of nicardipine) was prepared by heat compression. A mixture of copoly (lactic/glycolic acid, 900 mg) and nicardipine free base (100 mg) was dissolved in dichloromethane (10 mL). The dichloromethane was evaporated with a rotary evaporator, and the resultant mass was dried further under vacuum. The dried powder (40 mg) was charged into a Teflon tube (2 mm inner diameter). The tube was set in a stainless steel cylinder kept at 35 to 40°C. A pressure of 100 kg/cm^2 was applied between the upper and lower stainless steel dies. The compressed pellet was sterilized by γ irradiation (Nippon Shosha Service, Tokai, Ibaraki, Japan).

Patient Population and Management

Fifty consecutive patients were investigated in the Department of Neurosurgery, Tokyo Women's Medical University between October 1,1999, and December 31, 2001. The study was approved by the University Ethics Committee and informed consent was obtained by the principal investigator (H.K.). Table 65–1 lists the clinical aspects of the patients treated. Clinical grading used the World Federation of Neurological Surgeons scale.[5] Eligibility criteria were an admission cranial computed tomograph (CT) showing a Fisher grade 3 SAH[6] and early craniotomy for clipping of the aneurysm. Aneurysms of the internal carotid and middle cerebral arteries were approached through a frontotemporal craniotomy and aneurysms of the anterior communicating and distal anterior cerebral arteries were exposed via a midline frontal craniotomy. Nicardipine prolonged-release implants were placed in the cisterns of the internal carotid, middle cerebral [horizontal (M1), insular (M2), and/or opercular (M3) segments], and anterior cerebral [horizontal (A1), callosal (A2) and/or interhemispheric (A3) segments] arteries where thick clots existed, and therefore vasospasm related to delayed ischemic neurological deficits was highly likely to occur. The number of pellets and location of placement depended on the amount and site of subarachnoid clot on preoperative CT and on the location of the craniotomy necessary to clip the

TABLE 65–1 Characteristics of 50 Consecutive Patients with Thick Subarachnoid Hemorrhage

Characteristics		Number of Patients
Sex	Female/male	35/15
Age	<49	9
	50–59	12
	60–69	12
	>69	17
World Federation of Neurological Surgeons grade	1	21
	2,3	14
	4	9
	5	6
Subarachnoid blood on computed tomographic scan	Localized thick in sylvian fissure and/or interhemispheric fissure	16
	Diffuse thick	34
Ruptured aneurysm	Posterior communicating	15
	Middle cerebral	13
	Anterior cerebral	22
Day of surgery	0	13
	1	17
	2	6
	3	2
	4	2
Outcome on Glasgow outcome scale	Good recovery	28
	Moderate disability	12
	Severe disability	5
	Persistent vegetative state	3
	Dead	2

aneurysm. Cerebral vasospasm was assessed by angiography performed 7 to 12 days after SAH, incidence of delayed ischemic neurological deficits and low density areas on CT. Basic management of postoperative patients followed previously described procedures.[7]

Results

The number of implants utilized ranged from two (n = 6 patients) to 12 (n = 1, see Table 65–1). Delayed ischemic neurological deficits and low-density areas were seen in two patients. The remaining 48 patients did not develop delayed ischemic neurological deficits or low density areas on CT scan. On angiography performed 7 to 12 days after SAH, 40 patients had no or mild cerebral vasospasm. There were two patients with severe angiographic vasospasm that led to delayed ischemia and low density areas on CT. Eight patients had moderate vasospasm in arteries not adjacent to the nicardipine implants including arteries contralateral to a frontotemporal craniotomy, more distal to the exposed arteries, and arteries in the same cistern but not adjacent to the implants. Vasospasm did not develop in any artery that was adjacent to a nicardipine implant (Figs. 65–1 and 65–2). There were

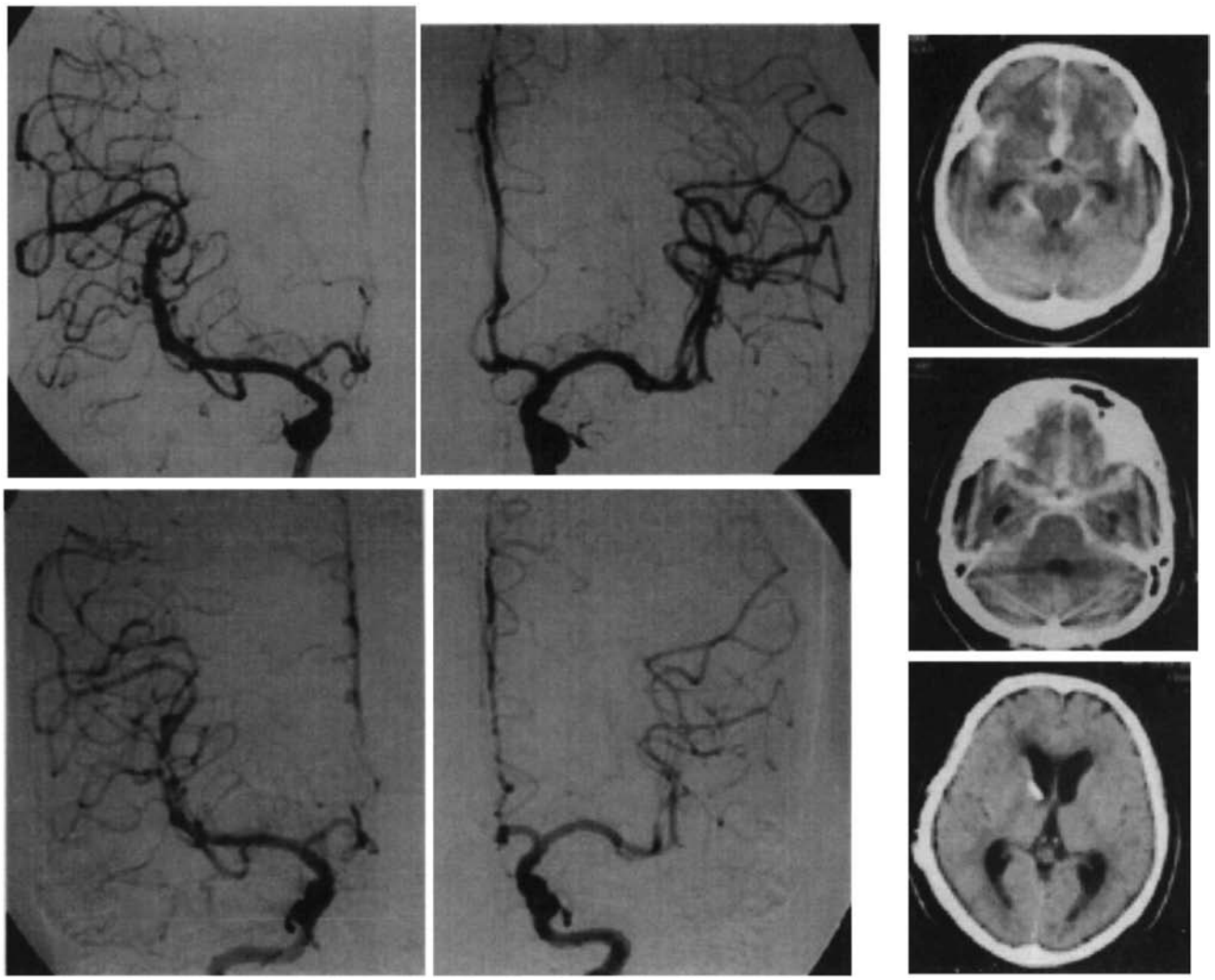


FIGURE 65–1 A 59-year-old female patient received nine nicardipine prolonged-release implants along both the anterior and the middle cerebral arteries (first and second segments of anterior cerebral, first segment of middle cerebral artery) after clipping of an anterior communicating artery aneurysm. Comparison of angiography before surgery (upper left and upper middle) and 10 days after SAH (lower left and lower middle) shows no vasospasm. Computed tomographic (CT) scan on admission (right upper, right middle) showed diffuse thick clot in the basal cisterns, suggesting a high risk for vasospasm. CT scan 30 days after SAH (lower right) did not show low-density areas due to cerebral vasospasm.

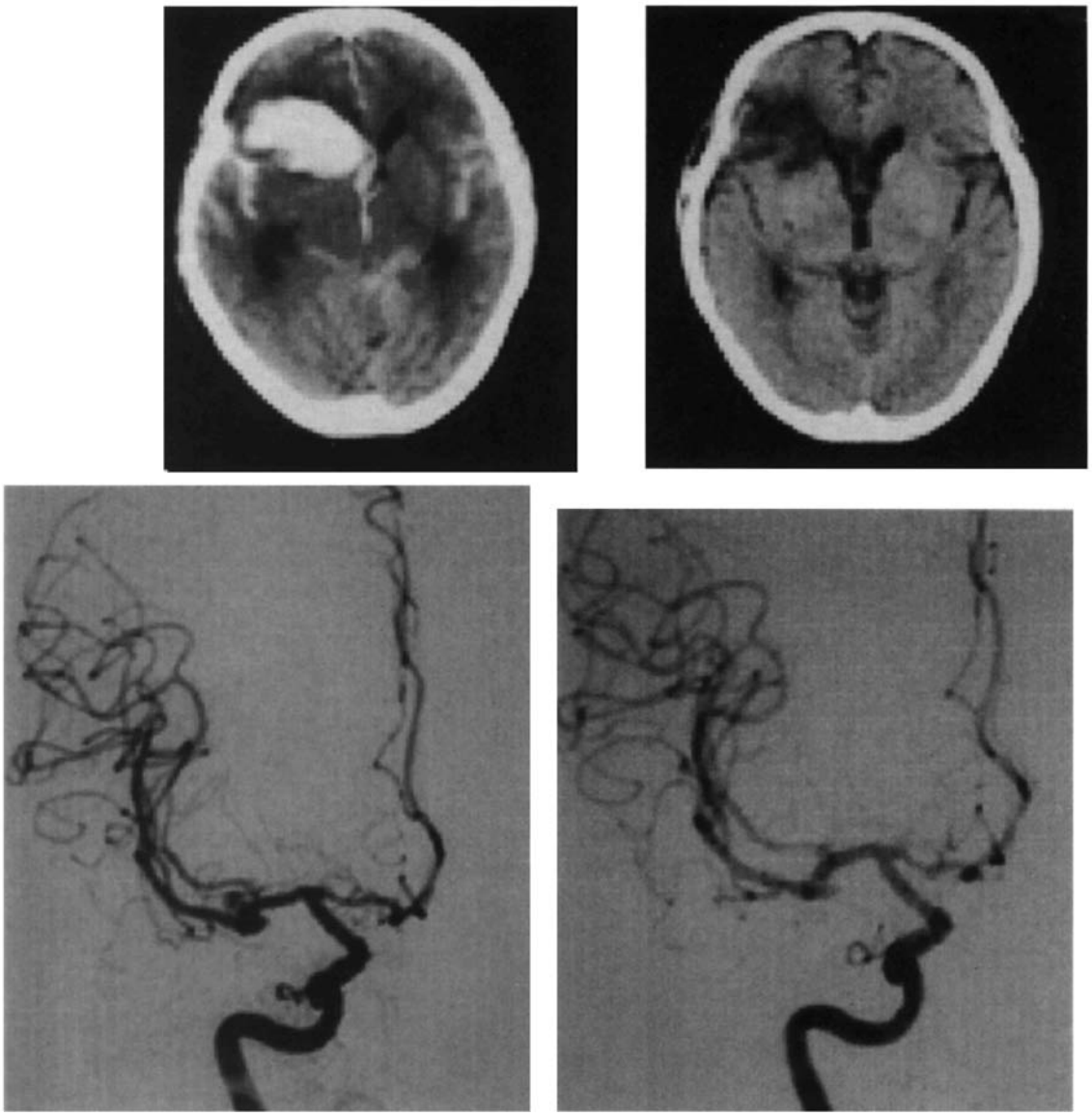


FIGURE 65–2 A 54-year-old female patient with localized thick clot in the right sylvian fissure (upper left) from a right middle cerebral artery aneurysm (preoperative angiography on lower left). Four nicardipine prolonged-release implants were placed adjacent to the first and second segments of the middle cerebral artery after clipping through a right frontotemporal craniotomy. There was no angiographic evidence of cerebral vasospasm on angiography 9 days later (lower right). There was a low-density area related to the hematoma on CT scan 33 days after subarachnoid hemorrhage (upper right).

no side effects in patients treated with this drug delivery system. Outcomes classified on the Glasgow outcome scale are shown in Table 65–1.[8] There were two deaths that were due to pulmonary embolism and pneumonia.

Discussion

We applied 2 to 12 implants (8 to 48 mg of nicardipine) into cisterns containing thick clot in patients with aneurysmal SAH and have demonstrated that this effectively prevents vasospasm in the arteries adjacent to the implants. The efficacy was less in arteries remote from the location of the implants. This was expected from our results in vitro that demonstrate high lipophilicity of nicardipine. Nicardipine was probably adsorbed to the clot and the arterial tissue near the implants due to its high lipophilicity and did not affect arteries at distant locations. Additional evidence for a very localized distribution of nicardipine was

that it was not detected in the cerebrospinal fluid of animals treated with implants after SAH.[9]

No side effects were noted in SAH patients treated with nicardipine implants. Our prior studies also did not detect neuronal toxicity in experimental models.[9] We stress that angiographic vasospasm did not appear in arteries in the cisterns with thick clots where vasospasm was expected to occur when pellets were placed adjacent to those arteries.[6] Therefore, vasospasm can be completely prevented in patients with localized thick clots. Although nicardipine implants may not be indicated in patients with diffuse thick clot, these implants could be placed in the proximal anterior cerebral artery, in the lower part of the second segment of the anterior cerebral artery, and along the first three segments of the middle cerebral artery on the side of a frontotemporal craniotomy. In addition, the first segments of the contralateral anterior and middle cerebral artery can be exposed and implants placed through a frontotemporal craniotomy. The interhemispheric approach allows placement of implants along the first two segments of the anterior and middle cerebral arteries bilaterally. When vasospasm in these parts of arteries is prevented, delayed ischemic deficits and/or infarction may not occur in most patients with diffuse thick clots.

Our results suggest that nicardipine can prevent vasospasm completely if an appropriate concentration of nicardipine is maintained in the surrounding cistern by using the newly developed drug-delivery system. This is probably explained on the basis that a high enough concentration of nicardipine might antagonize Ca^{2+} influx through receptor-operated as well as voltage-dependent Ca^{2+} channels.

REFERENCES

1. Kawashima A, Kasuya H, Shiokawa K, Miyajima M, Izawa M, Takakura K. Efficacy of nicardipine prolonged-release pellet on cerebral vasospasm in dogs. No Shinkei Geka 1998;26:37–43
2. Kawashima A, Kasuya H, Sasahara A, Miyajima M, Izawa M, Hori T. Prevention of cerebral vasospasm by nicardipine prolonged-release implants in dogs. Neurol Res 2000;22:634–641
3. Sasahara A, Kasuya H, Kawashima A, Aihara Y, Izawa M, Hori T. The efficacy and safety of the nicardipine prolonged-release implant in canine double hemorrhage model. No Shinkei Geka 2000;28:1071–1075
4. Shiokawa K, Kasuya H, Miyajima M, Izawa M, Takakura K. Prophylactic effect of papaverine prolonged-release pellets on cerebral vasospasm in dogs. Neurosurgery 1998;42:109–116
5. Drake CG, Hunt WE, Sano K, et al. Report of World Federation of Neurological Surgeons Committee on a Universal Subarachnoid Hemorrhage Grading Scale. J Neurosurg 1988;68:985–986
6. Fisher CM, Kistler JP, Davis JM. Relation of cerebral vasospasm to subarachnoid hemorrhage visualized by computerized tomographic scanning. Neurosurgery 1980;6:1–9
7. Kasuya H, Kawashima A, Namiki K, Shimizu T, Takakura K. Metabolic profiles of patients with subarachnoid hemorrhage treated by early surgery. Neurosurgery 1998;42:1268–1275
8. Jennett B, Bond M. Assessment of outcome after severe brain damage: a practical scale. Lancet 1975;1:480–484
9. Kasuya H, Onda H, Takeshita M, Okada Y, Hori T. Efficacy and safety of nicardipine prolonged-release implants for preventing vasospasm in humans. Stroke 2002;33:1011–1015

66

Transdermal Nitroglycerin in Patients with Subarachnoid Hemorrhage: A Pilot Study

MICHAEL REINERT, M.D., ROLAND WIEST, M.D., LORENCE BARTH, M.D., ROBERT ANDRES, M.D., CHRISTOPH OZDOBA, M.D., ROLF W. SEILER, M.D.

Abstract

Delayed ischemic neurological deficit due to cerebral vasospasm remains an important cause of morbidity and mortality in patients with subarachnoid hemorrhage (SAH). There is experimental and clinical evidence suggesting that nitric oxide donors may diminish cerebral vasospasm. We therefore analyzed the effect of transdermal nitroglycerin on transcranial Doppler (TCD) velocities, cerebral blood flow, and delayed ischemic deficits in patients with aneurysmal SAH. Nitroglycerin was administered transcutaneously in a dose of 14 µg/kg/hour. TCD was performed daily and cerebral blood flow measurements were done using the perfusion computed tomography technique. Blood pressure, volume intake, and vasopressor administration were recorded. Patients were randomly assigned to receive nitroglycerin (n = 9) or to serve as controls (n = 8). Mean TCD values in the extracranial portion of the internal carotid artery were lower in the group given nitroglycerin (p < .005). Mean TCD velocities in the middle cerebral arteries showed no differences between groups. The Lindegaard ratio was higher in the nitroglycerin group (p < .04). Cerebral blood flow was also higher in the patients given nitroglycerin compared with controls (p < .03). Even though nitroglycerin reduces blood pressure, lowers internal carotid artery TCD flow velocities, and increases the Lindegaard ratio, higher cerebral blood flows were measured in the patients exposed to nitroglycerin. Thus, nitroglycerin influences cerebrovascular tone and increases cerebral blood flow. Treatment of patients with SAH with nitroglycerin is possible without increasing the risk of delayed ischemic deficits. The exact timing of onset, duration, and dose of nitroglycerin in respect to the appearance of vasospasm may have a strong impact on the success of such a therapy.

Cerebral vasospasm is a major cause for morbidity and mortality in patients suffering from subarachnoid hemorrhage (SAH). After years of efforts in basic and clinical vasospasm research, these patients continue to have a 10 to 13% risk of ischemia and stroke from vasospasm despite nimodipine, hypertensive, hypervolemic, and hemodilution therapy.[1] Inhibition of vasodilatory pathways such as the nitric oxide (NO) pathway are presumed to be important events after SAH. Evidence for this includes the reduction in NO

synthase in the adventitia of spastic vessels.[2] Other studies have shown a high affinity of NO for oxyhemoglobin released from blood clots surrounding spastic vessels.[3,4] Reversal of vasospasm after SAH has been observed in studies where NO was administered either intravenously, intra-arterially or transdermally.[2,5–8]

The vasodilatory effect of nitroglycerin is related to NO release although the exact mechanism of this effect has been somewhat obscure. Chen et al[9] recently described the biotransformation of nitroglycerin, which occurs mainly in mitochondria through mitochondrial aldehyde dehydrogenase. Nitrite acts as an intermediate in generation of NO bioactivity from nitroglycerin, where nitrite is further processed by the electron transport chain (complex III and IV) and facilitates the transformation to NO.[10] NO then interacts with guanylate cyclase to form cyclic guanosine monophosphate, leading to relaxation of smooth muscle cells.[11] Prolonged administration of nitroglycerin classically is associated with clinical tolerance, which has been hypothesized to be related to attenuated biotransformation by mitochondrial aldehyde dehydrogenase.

The effect of increasing NO levels after SAH has been studied in animals and humans. In most of the studies done in animals, arterial diameter size has been used as a parameter for vasospasm[2,5–8,12–19] and an increase in arterial diameter has been observed after NO exposure. In a few clinical studies either intrathecally administered nitroprusside or intravenously administered nitroglycerin was used and the outcome measured usually was reduction in appearance of delayed ischemic neurological deficits or improvement in outcome. These encouraging results led us to study the effects of transdermal nitroglycerin in patients with SAH.

Materials and Methods

Study Groups, Nitroglycerin Dosage, Patient Management

The study was approved by the local ethics committee. Patients suffering from SAH with a clinical Hunt and Hess grade of 2 to 4 were included in the study. Informed consent was obtained. Patients were randomly assigned to treatment with nitroglycerin or to the control group. Patients in the control group received initial blood pressure control and aneurysm treatment within the first 24 hours of SAH. After treatment of the aneurysm, hemodynamic therapy was initiated. Nimodipine was given intravenously (2 mg/h). The group given nitroglycerin received, in addition to the preceding, transdermal nitroglycerin (Minitran, 3M Health Care, Pharmaceuticals, Abteilung Pharma, Switzerland) from days 1 to 9 after SAH. The target dose of nitroglycerin was 14 µg/kg/h. On day 8, nitroglycerin was reduced to 10 µg/kg/h, on day 9 to 7 µg/kg/h, and on day 10 nitroglycerin was stopped. To prevent arterial hypotension in patients receiving nitroglycerin, a standard algorithm was used consisting of increasing volume with 6% hydroxyethyl starch (Voluven) in 500 mL aliquots, reduction of nimodipine by 0.5 mg/h steps, and finally, by additional noradrenalin.

Transcranial Doppler, Cerebral Blood Flow, and Clinical End Points

Daily transcranial Doppler (TCD) studies (DWL Multi-DOP, Uberlingen, Germany) were performed by the same person for 10 days. Measurements of flow velocities were made in the extracranial portion of the internal carotid, the middle cerebral artery, and the anterior cerebral and posterior cerebral arteries. Middle cerebral artery flow velocities > 120 cm/s and a Lindegaard ratio > 3 were considered to represent TCD vasospasm. The Lindegaard ratio was defined as the flow velocity in the middle cerebral artery over the flow velocity in the extracranial internal carotid artery.[20] The examiner was blinded to the study group.

Cerebral blood flow was assessed using a a multislice computed tomographic (CT) scanner (LightSpeed, General Electric, Milwaukee, WI, USA). Studies were performed according to a standardized protocol that allowed measurement of two parallel 10 mm sections with a temporal resolution of 1 second. A nonionic contrast agent (Iomeprol 400, Bracco, SA, Milan, Italy) was injected intravenously at a flow rate of 4 mL/sec. Regional cerebral blood flow values were calculated with a commercially available program (Advantage Windows CT Perfusion, General Electric, Milwaukee, WI, USA). The neuroradiologists performing these studies were blinded to results from other modalities. Regional blood flow values were determined for standardized areas in the territories of the anterior, middle, and posterior cerebral arteries in both hemispheres.

New neurological deficits such as hemiparesis, cranial nerve palsy, loss of orientation, aphasia, and decrease in consciousness were considered as clinical vasospasm (delayed ischemic neurological deficit). Patients were assessed twice a day during the monitoring period.

Statistical Analysis

Mean daily blood pressure, volume intake, and vasopressor application were used for statistical analysis. The χ-square, unpaired, one-tailed Mann-Whitney-U and paired Wilcoxon tests were used. Significance was set at $p < .05$.

Results

Seventeen patients were randomized to receive nitroglycerin (n = 9) or to the control group (n = 8). The aneurysm locations were evenly distributed in both groups. The clinical grading according to Hunt and Hess as well as Fisher grading were not significantly different. There was a trend for higher Fisher grades in the nitroglycerin group. Systolic and diastolic blood pressure differed significantly ($p < .005$). Mean systolic blood pressure was 140 mmHg in the nitroglycerin group compared with 147 mmHg in the control group. Mean diastolic blood pressure was 74 mmHg in the nitroglycerin group and 79 mmHg in the control group. There were no significant differences in volume intake (mean value of 3710 mL in the nitroglycerin compared with 3432 mL in the control group) and vasopressor use between the groups.

The TCD flow velocities in the extracranial portion of the internal carotid arteries were significantly lower in the nitroglycerin group ($p < .05$, Fig. 66–1). On the other hand, TCD flow velocities in the middle, anterior, and posterior cerebral arteries for right and left sides did not differ significantly between groups (Fig. 66–2). The Lindegaard ratio was significantly higher in the patients treated with nitroglycerin ($p < .04$, Fig. 66–3). There was no significant difference between the two groups in clinical appearance of delayed ischemic neurological deficits.

Cerebral blood flow studies were performed in 12 patients (eight in the nitroglycerin and four in the control group). The blood flow values in the nitroglycerin group were significantly higher than in the control group ($p < .03$, 42 ± 3 vs 32 ± 4, Fig. 66–4). The difference in cerebral blood flow values in patients with compared with those without delayed ischemic neurological deficits was not significant although there was a trend toward lower values in patients with deficits ($p = .068$). The cerebral blood flow values in patients with vasospasm based on TCD values were not different from those without vasospasm on TCD.

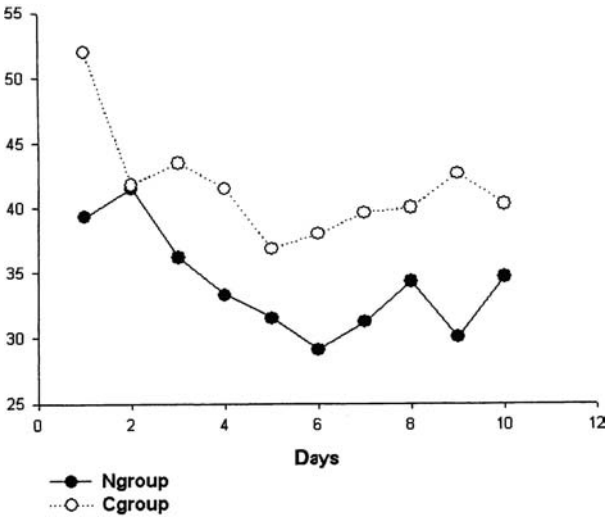


FIGURE 66–1 Mean transcranial Doppler flow velocities in the extracranial portion of the internal carotid artery by day after subarachnoid hemorrhage. Values on y-axis are mean flow velocity in cm/sec. The values in the group treated with nitroglycerin (Ngroup) were significantly lower than those in the control group. (Cgroup; $p < .003$).

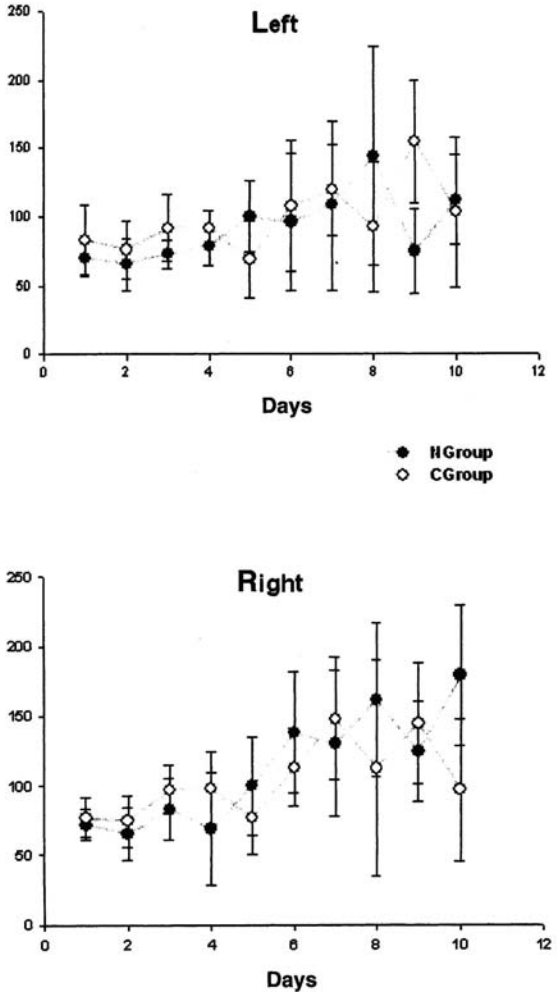


FIGURE 66–2 Mean transcranial Doppler flow velocities in the left and right middle cerebral arteries by day after subarachnoid hemorrhage for each group. Values on y-axis are mean flow velocity in cm/sec. No significant differences were noted between the group receiving nitroglycerin (Ngroup) and untreated controls (Cgroup).

Discussion

Data reported thus far concerning the effect of NO donors on vasospasm after SAH have not been

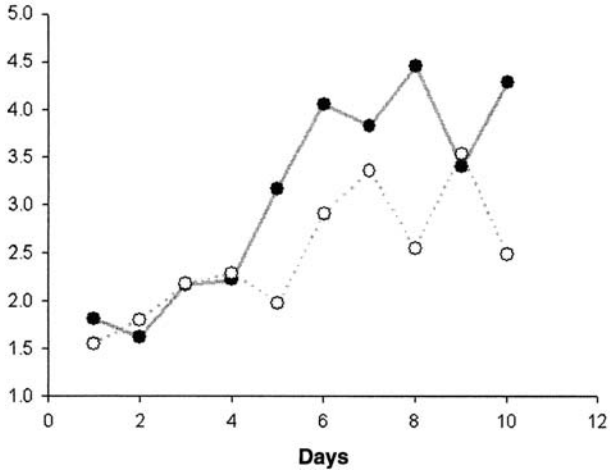


FIGURE 66–3 Mean Lindegaard ratio by day after subarachnoid hemorrhage for each group. A significant difference ($p < .04$) was found between the patients treated with nitroglycerin (solid circles) and controls circles (open circles).

conclusive. However, the results of these studies have been rather encouraging. Nitroglycerin is an NO donor that has been used clinically for years. Its mechanism of action has only recently been described.[9,21] The main systemic risk is hypotension and this is known to occur in a dose-dependent fashion. There is evidence that nitroglycerin has an effect on cerebral blood vessels and blood flow.[7,8,13,14,16,18,19,22,23] Nitroglycerin increased cerebral blood flow, reduced cerebral vasospasm, and reduced the clinical occurrence of

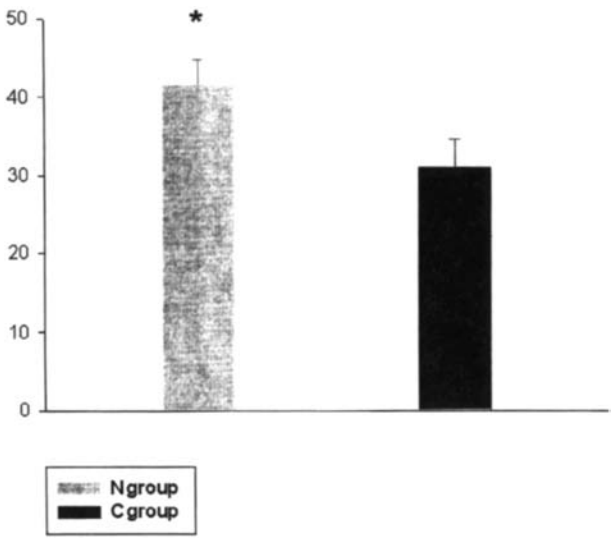


FIGURE 66–4 Mean cerebral blood flow measured using perfusion computed tomography. The cerebral blood flow was significantly higher in the group treated with nitroglycerin (Ngroup) compared with the untreated controls (Cgroup 42 ± 3 vs 32 ± 4, $p < .03$).

delayed ischemic neurological deficits in clinical trials as well as in experimental studies. The increased cerebral blood flow values found in the present study support these findings. The occurrence of clinical vasospasm in our study, however, was not significantly different between groups.

Tolerance to nitroglycerin has been well described and may be related to alterations in mitochondrial aldehyde dehydrogenase.[9,21] Tolerance remains a potential problem because prolonged treatment for days may be required in patients at risk for vasospasm. There is also the issue of rebound of vasospasm after reduction of nitroglycerin. Arterial hypotension is another potential risk in the application of nitroglycerin. We have found in our study that systolic and diastolic blood pressure were significantly lower in patients treated with nitroglycerin compared with controls. Higher intravenous fluid volumes were required after administration of nitroglycerin. It has been suggested that nitroglycerin be administered with vasopressors to counteract hypotension.[12] The increased Lindegaard ratio reported herein is, in our view, a result of this decrease in blood pressure. Interestingly cerebral blood flow seems not to be negatively affected because an increase in cerebral blood flow occurred in patients treated with nitroglycerin.

There are no specific data on whether nitroglycerin induces the production of free radicals and lipid peroxidation but it is generally known that other NO donors or NO synthase activators can enhance production of the substances.[11] Nitroglycerin might have obtained a more favorable role in treatment of patients with SAH had there not been potential risks, such as hypotension, which is so adamantly avoided in SAH patients.

Conclusion

This small study suggests that transdermal nitroglycerin positively influences cerebral blood flow. Nitroglycerin therapy is possible without increasing the risk of clinical vasospasm. Induced hypervolemia and possibly more liberal use of vasopressors together with nitroglycerin may further reduce the risk of delayed cerebral ischemia after SAH. Yet optimal starting time and the duration of nitroglycerin therapy have to be defined. *Dosis et tempus faciunt successum.*

Acknowledgment

3M Pharmaceuticals Switzerland, provided the Minitran at no expense. There was no financial interest or conflict with the study protocol or publication of the study data. We acknowledge Nadine Reinert for the Latin adaptation of Paracelsus's phrase *Dosis facit venerium.*

REFERENCES

1. Lanzino G, Kassell NF, Dorsch NW, et al. Double-blind randomized, vehicle-controlled study of high-dose tirilazad mesylate in women with aneurysmal subarachnoid hemorrhage, I: A cooperative study in Europe, Australia, New Zealand and South Africa. J Neurosurg 1999;90:1011–1017
2. Pluta RM, Oldfield EH, Boock RJ. Reversal and prevention of cerebral vasospasm by intracarotid infusions of nitric oxide donors in a primate model of subarachnoid hemorrhage. J Neurosurg 1997;87:746–751
3. Iadecola C, Pelligrino DA, Moskowitz MA, et al. Nitric oxide synthase inhibition and cerebrovascular regulation. J Cereb Blood Flow Metab 1994;14:175–192
4. Vane JR, Anggard EE, Botting RM. Regulatory functions of the vascular endothelium. N Engl J Med 1990;323:27–36
5. Afshar JK, Pluta RM, Boock RJ, et al. Effect of intracarotid nitric oxide on primate cerebral vasospasm after subarachnoid hemorrhage. J Neurosurg 1995;83:118–122
6. Ito Y, Mizuno Y, Azuma H, et al. Effective improvement of the cerebral vasospasm after subarachnoid hemorrhage with low-dose nitroglycerin. J Cardiovasc Pharmacol 2000;35: 45–50
7. Tanaka Y, Masuzawa T, Saito M, et al. Combined administration of fasudil hydrochloride and nitroglycerin for treatment of cerebral vasospasm. Acta Neurochir Suppl 2001;77: 205–207
8. Thomas JE, Rosenwasser RH, Armonda RA, et al. Safety of intrathecal sodium nitroprusside for the treatment and prevention of refractory cerebral vasospasm and ischemia in humans. Stroke 1999;30:1409–1416
9. Chen Z, Zhang J, Stamler JS. Identification of the enzymatic mechanism of nitroglycerin bioactivation. Proc Natl Acad Sci USA 2002;99:8306–8311
10. Kozlov AV, Staniek K, Nohl H. Nitrite reductase activity is a novel function of mammalian mitochondria. FEBS Lett 1999; 454:127–130
11. Macdonald RL, Weir B. Cerebral Vasospasm. San Diego: Academic; 2001:1–518
12. Essen C, Kistler P, Lees RS, et al. Cerebral blood flow and intracranial pressure in the dog during intravenous infusion of nitroglycerin alone and in combination with dopamine. Stroke 1981;12:331–338
13. Frazee JG, Giannotta SL, Stern WE. Intravenous nitroglycerin for the treatment of chronic cerebral vasoconstriction in the primate. J Neurosurg 1981;55:865–868
14. Heros RC, Zervas NT, Lavyne MH, et al. Reversal of experimental cerebral vasospasm by intravenous nitroprusside therapy. Surg Neurol 1976;6:227–230
15. Kanamaru K, Weir BK, Findlay JM, et al. Pharmacological studies on relaxation of spastic primate cerebral arteries in subarachnoid hemorrhage. J Neurosurg 1989;71:909–915
16. Kistler JP, Lees RS, Candia G, et al. Intravenous nitroglycerin in experimental cerebral vasospasm: a preliminary report. Stroke 1979;10:26–29
17. Macdonald RL, Zhang ZD, Curry D, et al. Intracisternal sodium nitroprusside fails to prevent vasospasm in nonhuman primates. Neurosurgery 2002;51:761–768
18. Poletti CE, Wepsic JG, Sweet WH. Middle cerebral artery spasm from subarachnoid blood: spasmolysis with topical use of nitroglycerin. Surg Forum 1972;23:449–450
19. Raabe A, Zimmermann M, Setzer M, et al. Effect of intraventricular sodium nitroprusside on cerebral hemodynamics and oxygenation in poor-grade aneurysm patients with severe, medically refractory vasospasm. Neurosurgery 2002;50:1006–1013
20. Lindegaard KF, Nornes H, Bakke SJ, et al. Cerebral vasospasm after subarachnoid hemorrhage investigated by means of transcranial Doppler ultrasound. Acta Neurochir Suppl (Wien) 1988;42:81–84
21. Innarro LJ. After 130 years, the molecular mechanism of action of nitroglycerin is revealed. Proc Natl Acad Sci USA 2002; 99:7816–7817
22. Nakao K, Murata H, Kanamaru K, et al. Effects of nitroglycerin on vasospasm and cyclic nucleotides in a primate model of subarachnoid hemorrhage. Stroke 1996;27:1882–1887
23. Sasanuma J, Ogayama H, Sasaki J, et al. Clinical effect of nitroglycerin (GTN) on prevention of cerebral vasospasm. No Shinkei Geka 1991;19:227–232

67

Decision Analysis in the Treatment of Vasospasm After Aneurysmal Subarachnoid Hemorrhage

CHARLES J. PRESTIGIACOMO, M.D., JEFFREY FARKAS, M.D., C. DAVID HUNT, M.D.

Abstract

Cerebral vasospasm is recognized as a potentially lethal complication of subarachnoid hemorrhage. With the introduction of hypervolemic, hypertensive, hemodilution (hemodynamic) therapy and angioplasty, numerous case series suggest that the overall incidence of cerebral infarction due to vasospasm is reduced when compared with historical data. There are few randomized trials comparing the use of hemodynamic therapy with angioplasty or medical management alone. Decision analysis is a method that can be used to evaluate these treatments and compare outcomes in a variety of settings with regard to numerous outcome measures. A clinical decision tree was created for the treatment of clinical vasospasm including (1) conservative management alone, (2) hemodynamic therapy, (3) hemodynamic therapy followed by angioplasty if there is failure of hemodynamic therapy, and (4) angioplasty. A review of the pertinent literature was performed to obtain probability ranges for variables involved in the determination of outcome, which include the complication rates and success and failure rates for hemodynamic therapy and angioplasty, including poor neurological outcome and death. Outcomes were measured according to quality-adjusted life expectancy (QALE). One-way and two-way sensitivity analysis was performed to determine the stability of the model algorithms as well as to help determine threshold values where one modality of treatment may be favored over another. Based on available studies, conservative management demonstrates a uniformly lower QALE than either hemodynamic therapy or angioplasty. Furthermore, based on the published complication rates and success rates for each modality, the model suggests a slightly higher QALE (~2 years) when angioplasty is employed earlier in the algorithm when compared with hemodynamic therapy alone or a combination of hemodynamic therapy and angioplasty in the setting of hemodynamic therapy failure. Though seemingly small, such values have been deemed to be clinically relevant. Sensitivity analysis was then performed, assessing for the stability of the model with variations in several parameters (such as complication rates for angioplasty and hemodynamic therapy and their respective outcomes). These results suggest better outcomes for hemodynamic therapy only if complication rates for angioplasty are somewhat greater than the published literature and if hemodynamic therapy carries a very low complication rate matched with a very high success rate. Decision analysis is a

powerful mathematical tool that can allow comparison of differing treatment modalities for various disease entities through the incorporation of published results and ranges of outcomes. Its potential utility is demonstrated in the current analysis of data collected from case series. Further analysis of the model is required to determine how subgroup analysis based on timing or initial neurological grade might affect these outcomes.

The lethality of vasospasm-induced delayed ischemic deficits secondary to aneurysmal subarachnoid hemorrhage (SAH) cannot be denied. Scientists and clinician-scientists continue to search for the etiology of vasospasm in an attempt to reduce its incidence and ultimately improve the overall outcome for patients sustaining aneurysmal SAH. At present, treatment algorithms for clinical vasospasm include various combinations of hypervolemia, hypertension, and hemodilution (hemodynamic) therapy. In 1984, Zubkov introduced a novel approach to the treatment of intracranial vasospasm by performing balloon angioplasty of the affected vessels.[1] Numerous case series suggest that the overall incidence of permanent delayed ischemic deficits is reduced by this procedure, when compared with historical data. However, hemodynamic therapy and balloon angioplasty have not been validated by randomized controlled trials. Strategies that would ultimately integrate these complementary and potentially competing modalities of treatment and most importantly, maximize the probability of a good outcome for each individual, are essential.

Clinical decision analysis, a mathematical tool that enables investigators to analyze simulated clinical situations using published data and estimates is a strategy that clinicians have used to help maximize treatment algorithms for certain disease states. This study compares the use of hemodyamic therapy, angioplasty, and conservative management in clinical vasospasm through the use of decision analysis.

Methods

A decision tree was created for the treatment of clinical vasospasm with the following treatment options: (1) conservative management alone, (2) hemodynamic therapy, (3) hemodynamic therapy followed by angioplasty if there is failure of hemodynamic therapy, and (4) angioplasty (Fig. 67–1). A review of the pertinent literature was performed to obtain probability ranges for variables involved in the determination of outcome, which include the complication rates and success and failure rates for hemodynamic therapy and angioplasty, including excellent and poor neurological outcomes and death (Table 67–1).[2–8] Outcomes were measured according to a simple relative utility scale. The results were then mathematically calculated by combining the probabilities of certain events occurring with the expected relative utilities. The limb of the decision tree with the maximum expected outcome is the treatment algorithm of choice for that particular clinical situation.

To determine the validity of the decision tree's conclusion, also known as the model's stability, several analyses were performed on the model. The "standard deviation" of a treatment algorithm suggests the risk of an adverse outcome if that limb of the tree is chosen. Lower values are equivalent to lower risk. Comparative probability distributions enable investigators to compare the risks over the entire probability range. Sensitivity analysis was employed to test whether the conclusions are similar if probabilities for certain variables are changed within plausible ranges. In addition, such analyses can be used to determine values at which the optimal strategy for a given scenario changes (threshold values).

Results

Extensive literature review resulted in a preponderance of Class 4 data (studies whose conclusions are based on case series with or without historical control data). One study however, did evaluate the efficacy of hemodynamic therapy by applying meta-analysis.[2] Based on available studies, conservative management demonstrates a uniformly lower utility than either hemodynamic therapy or angioplasty, suggesting that conservative measures in the treatment of clinical vasospasm is not the preferred treatment modality.

In assessing the utility values for angioplasty and hemodynamic therapy, the difference in relative utility scores is small (~0.03). In view of the published data, the model suggests that the use of angioplasty as a first option is preferred. In addition, in the clinical scenario where there is failure of hemodynamic therapy, the model suggests that the patient might benefit from angioplasty at that stage instead of undergoing further, more aggressive forms of hemodynamic therapy.

Analysis of the results demonstrated a standard deviation of 0.357 for angioplasty and 0.358 for

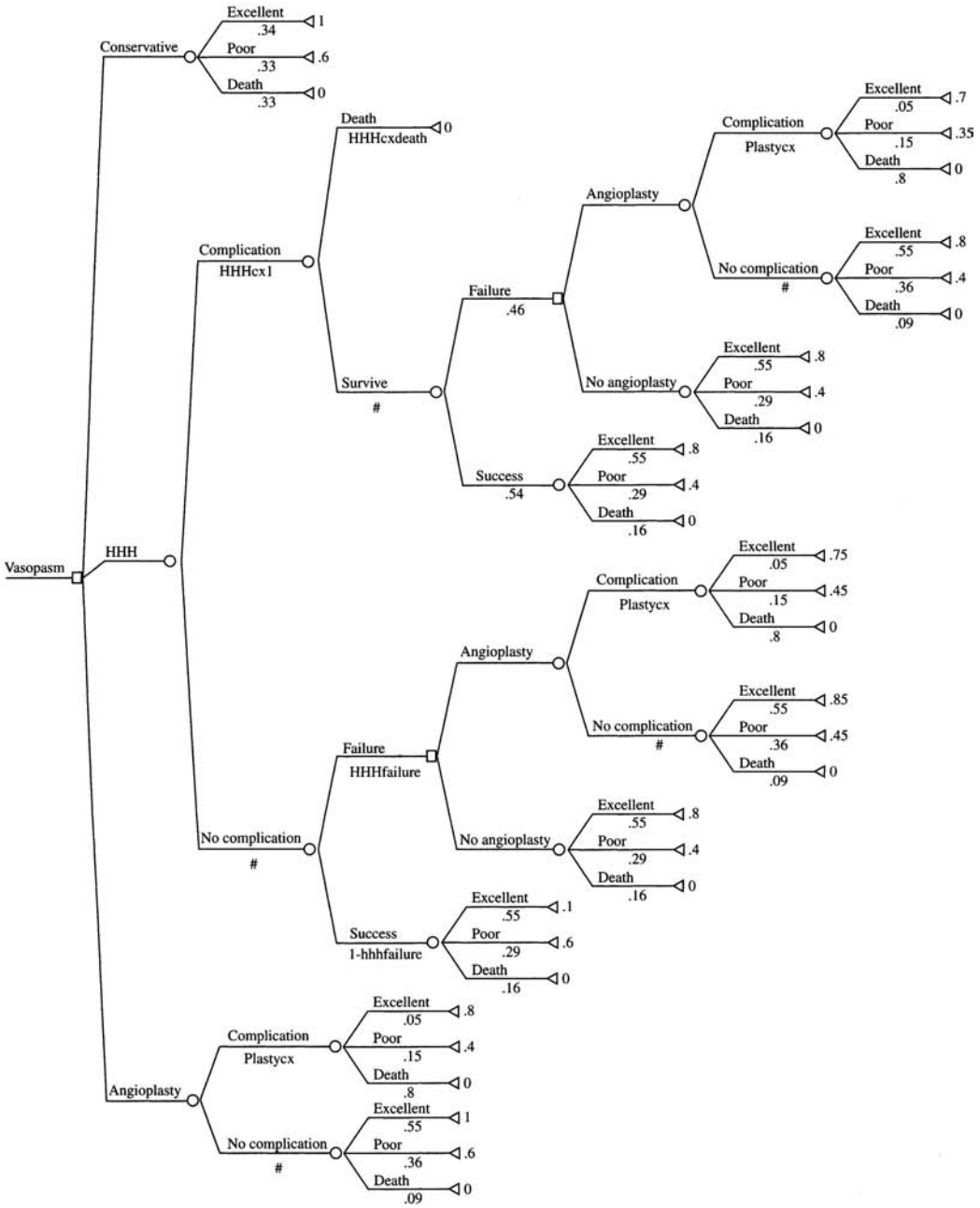


FIGURE 67–1 Final, fully expanded decision tree. Squares represent decision nodes, circles represent chance nodes, and triangles represent terminal nodes. Values at end of each limb represent relative utility and values beneath each limb represent relative probabilities for the labeled event (hhhcx: hemodynamic therapy complication, plastycx: angioplasty complication).

hemodynamic therapy. This finding suggests that there is a tendency for angioplasty to carry less risk of an adverse outcome when compared with hemodynamic therapy. This finding is confirmed by assessing the probability distribution curves comparing the two modalities (Fig. 67–2). Two-way sensitivity analysis assessing the model's stability in the setting of varying the complication rates for angioplasty and hemodynamic therapy within specific ranges (4–25% and 5–35%, respectively), demonstrated a higher utility in performing angioplasty over hemodynamic therapy (Fig. 67–3). According to the model and the published data, hemodynamic therapy would have a higher expected utility (and thus be the preferred modality of treatment) if complication rates for angioplasty were > 19% and the complication rate of hemodynamic therapy was between 5 and 25%.

TABLE 67–1 Values Used for Decision Analysis and Literature Source

Treatment	Treatment Variable	Reference	Value (Plausible Range Used)
Conservative	Outcome	Dorsch[3]	Good/excellent in 34% Poor in 33% Death in 33%
Hemodynamic therapy	Complication rate	Origitano et al[5]	20% (5–35%)
	Success rate	Pritz et al[2]	54%
	Outcomes	Dorsch and King[6] Macdonald[4]	Good/excellent in 55% Poor in 29% Death in 16%
Angioplasty	Complication rate	Newell et al[7] Eskridge et al[8]	10% (4–25%)
	Outcomes	Newell et al[7] Eskridge et al[8]	Good/excellent in 55% Poor in 36% Death in 9%

Discussion

This decision model applies rigorous mathematical principles to the determination of the best treatment algorithm for vasospasm. Importantly, when applying the published data to this model, the use of angioplasty as either an adjunct or the primary means of treating vasospasm is preferable to hemodynamic therapy alone. Most importantly, the difference between the two outcomes is small though significant. Such a difference can translate into a clinically relevant quality-adjusted life expectancy (QALE). QALE assesses the patient's preference for the quality of life in a specific outcome state. For example, a patient may prefer 2 years with no morbidity over 10 years with significant morbidity. In other words, the usefulness or utility for a given outcome state (recovery, disability, death) for a given patient should be included in the final analysis of outcome and is calculated using the following equation: QALE = LE × U where LE is the life expectancy for the given age of the patient as determined by life tables and U is the utility for that given outcome state. Sensitivity analysis is quite important in this study in that it clearly

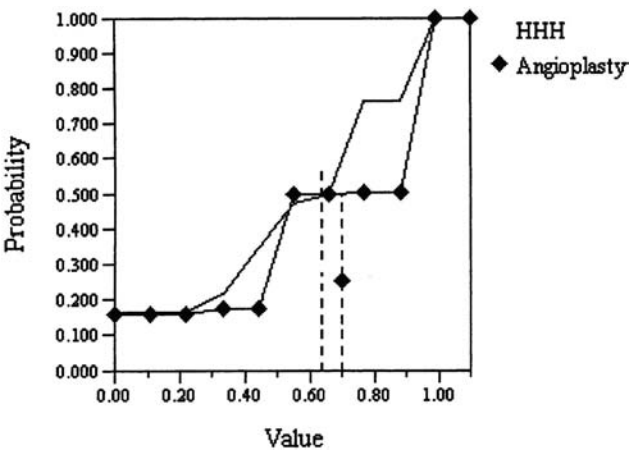


FIGURE 67–2 Comparative probability distribution. Solid line is hemodynamic therapy (HHH) and line with diamonds is angioplasty. X-axis represents the value of the procedure and *y*-axis is probability of an adverse event. There is a trend for a higher expected value with a lower probability of an adverse event for angioplasty when compared with hemodynamic therapy.

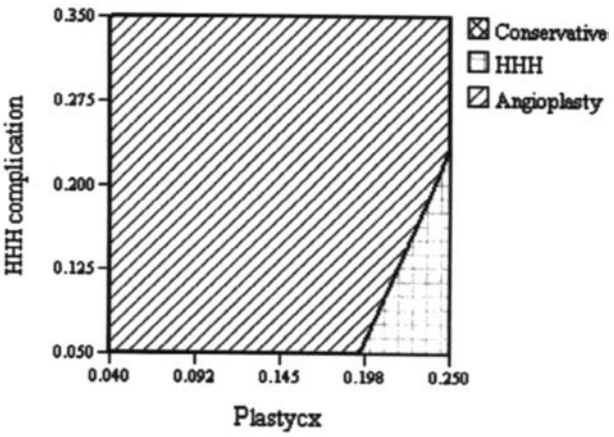


FIGURE 67–3 Two-way sensitivity analysis. This graph compares complication rates for hemodynamic (HHH) therapy and angioplasty (plastycx). Note that angioplasty (field with diagonal lines) has better outcome values up to the point where the complication rate for angioplasty exceeds 19% (0.19), provided that complication rates for hemodynamic therapy do not exceed 5%. As the complication rate for angioplasty increases, the "threshold value" for hemodynamic therapy (i.e., the acceptable complication rate for hemodynamic therapy) can increase (cross-hatched area of the graph).

demonstrates this small benefit in angioplasty for a broad range of values.

Several limitations to this study do exist. First, the potential for literature bias exists because the true complication rates for hemodynamic therapy and angioplasty may be higher. Sensitivity analysis can compensate for this by applying the model to broad, plausible ranges as was done in this study. Most importantly, the model cannot account for the intrinsic biases that exist within the studies chosen for this analysis. The strength of decision analysis rests on the studies available at the present time. The studies used in this analysis were retrospective and observational and therefore open to significant study design biases. Decision analysis does not negate the need for randomized control trials, but rather it points to areas where the need is likely to be greatest.

Conclusion

This study demonstrates a role for balloon angioplasty in the setting of clinical vasospasm. However, given the relative lack of prospective data on the risks and benefits of hemodynamic therapy and angioplasty, these conclusions cannot be as yet generalized to all patients. The observation presented here that differences are small suggests a need to study both modalities not only as complementary treatments but also to examine balloon angioplasty as a first-line treatment, perhaps in patients where the risk of hemodynamic therapy is relatively high.

REFERENCES

1. Zubkov YN, Nikiforov BM, Shustin VA. Balloon catheter technique for dilatation of constricted cerebral arteries after aneurysmal SAH, Acta Neurochir 1984;70:65–79.
2. Pritz MB, Zhou XH, Brizendine EJ. Hyperdynamic therapy for cerebral vasospasm: a meta-analysis of 14 studies. J Neurovasc Dis 1996;1:6–8
3. Dorsch NWC. Incidence, effects and treatment of ischemia following aneurysm rupture. In: Sano K, Takakura K, Kassell NF, Sasaki T, eds. Cerebral Vasospasm. Tokyo: Univeristy of Tokyo Press; 1990:495–498
4. Macdonald RL. Cerebral vasospasm. In: Welch KM, Caplan LR, Reis DJ, Siesjo BK, Weir B, eds. Primer on Cerebrovascualr Diseases. San Diego: Academic; 1997:490–497
5. Origitano TC, Wascher TM, Reichman OH, Anderson DE. Sustained increased cerebral blood flow with prophylactic hypertensive hypervolemic hemodilution ("Triple-H" therapy) after subarachnoid hemorrhage. Neurosurgery 1990;27:729–740
6. Dorsch NWC, King MT. A review of cerebral vasospasm in aneurysmal subarachnoid hemorrhage, I: Incidence and effects. J Clin Neurosci 1994;1:19–24
7. Newell DW, Eskridge JM, Mayberg MR, Grady MS, Winn HR. Angioplasty for the treatment of vasospasm following subarachnoid hemorrhage. J Neurosurg 1989;71:654–660
8. Eskridge JM, McAuliffe W, Song JK, et al. Balloon angioplasty for the treatment of vasospasm. Neurosurgery 1998;42:979–986

68

Effects of Fasudil Hydrochloride, a Protein Kinase Inhibitor, on Cerebral Vasospasm and Infarction

MASATO SHIBUYA, M.D., SKN-ICHI SATOH, AND YOSHIO SUZUKI, M.D.

Abstract

Fasudil hydrochloride is used in Japan in the majority of patients with subarachnoid hemorrhage (SAH) to ameliorate vasospasm. A recent Chinese clinical study also suggested its superiority to nimodipine (M. Shibuya, unpublished observations). Its effect consists of both vasodilation and brain protection from ischemia. It inhibits protein kinases (Rho kinase, protein kinase C, and myosin light chain kinase) that are known to be involved in the pathophysiology of vasospasm and brain infarction. A placebo-controlled, double-blind study of the effect of fasudil was performed on 160 patients who had motor weakness caused by acute cerebral infarction due to arterial thrombosis. Fasudil HC1, 60 mg, or saline (2 mL), dissolved in 100 mL of saline was given intravenously over 60 minutes two times a day for 14 days. Patients were examined neurologically and by either or both cranial computed tomography and magnetic resonance imaging. Outcome was examined at 1 month after the onset. Fasudil significantly decreased deterioration of both motor function and activities of daily living. Motor dysfunction improved in 30% of treated patients compared with 19% of controls and dysfunction worsened in 3% and 18%, respectively ($p < .025$). Activities of daily living improved in 63% of treated patients and 44% of controls and deteriorated in 1% and 12%, respectively ($p < .025$, Wilcoxon test). There were no differences between groups in side effects. These results suggest that fasudil exerts neuroprotective effects in acute cerebral infarction. Inhibition of Rho kinase and protein kinase C may therefore be important in ameliorating vasospasm and in reducing infarction in patients with SAH.

Fasudil [hexahydro-1-(5-isoquinolinesulfonyl)-1H-1,4-diazepine hydrochloride or AT877, HA1077, Eril] is a unique vasodilating and brain protecting drug. It dilates spastic arteries when given intravenously without causing significant hypotension.[1] It has been used in Japan since 1995 for prevention of vasospasm in the majority of patients with aneurysmal subarachnoid hemorrhage (SAH). A recent Chinese clinical comparative study on the effect of fasudil and nimodipine in patients with SAH has also confirmed its efficacy. In these studies fasudil showed more brain protective effect than vasodilating effect, suggesting that it might exhibit efficacy in the treatment of cerebral infarction. A recent double-blind trial of fasudil on patients with acute cerebral infarction in Japan showed that it significantly improved both motor dysfunction and activities of daily living. Multiple mechanisms of action of fasudil have been elucidated, but the main effects are believed to be

mediated by inhibition of protein kinases including Rho kinase, protein kinase C, and myosin light chain kinase.

Fasudil Hydrochloride

Fasudil is water soluble and relatively resistant to light. It has a Ca^{2+} antagonistic effect (pA2 = 6.71).[2] It has been shown to inhibit various protein kinases including myosin light chain kinase (K_i = 36 μmol/L), protein kinase C (K_i = 3 μmol/L), and Rho kinase (K_i - 0.3 μmol/L, Table 68–1).[3] It dilates cerebral arteries more than systemic arteries, which is important to avoid hypotension. It dilated spastic arteries in a double-hemorrhage canine model when administered intravenously.[1] Biological half-life of intravenously administered fasudil is < 30 minutes. However, its hydroxylated metabolite (M3) also dilates spastic arteries with a half-life that is as long as 8 hours. M3 also inhibits protein kinases with K_i values for myosin light chain, protein kinase C, and Rho kinase of 140,18, and 0.18 μmol/L, respectively.[4] These data showing that M3 has a longer half-life and is a potent Rho kinase inhibitor suggest that part of the effect of fasudil may depend on M3.

Effect of Fasudil on Human Vasospasm

Fasudil has been widely used in Japan. The effects of fasudil on patients with SAH treated after the drug was approved in Japan have been compared with those obtained in the double-blind trial and the results are similar.[5] Fasudil significantly decreased symptomatic vasospasm in patients with Fisher grade 3 and 4 SAH on computed tomography (CT).[6] It significantly decreased development of angiographic vasospasm and significantly improved patient outcome in Fisher group 3.[7] Intra-arterial use of fasudil also effectively dilates vasospastic arteries. It is common practice to administer 30 mg of fasudil during follow-up angiography after aneurysmal SAH through the angiography catheter. This should be given in divided doses with close monitoring of blood pressure (Fig. 68–1). The vasodilating effect of intra-arterial fasudil is as effective as that of papaverine but there are fewer side effects than are reported with papaverine. This may make fasudil superior to papaverine for intra-arterial use.[8]

Effects of Fasudil on Cerebral schemia

Fasudil has been shown to have neuroprotective effects in both gerbil and rat models of cerebral ischemia. Fasudil (3 mg/kg intraperitoneally twice a day for 7 days) significantly inhibited loss of hippocampal CA1 neurons following bilateral carotid artery occlusion for 5 minutes in gerbils.[9] Regional cerebral blood flow was measured by ^{14}C-iodoantipyrine and regional glucose use by ^{14}C-2-deoxyglucose 7 days after bilateral carotid occlusion in rats.[10] Both parameters were reduced compared with control rats and fasudil (3 mg/kg intraperitoneally) significantly increased blood flow and glucose use. Similar brain protection by fasudil has been shown in other models of ischemia induced by lauric acid[11] and glass microspheres in rats.[12]

Blood viscosity was increased from 5.3 to 6.2 centipoise 24 hours after a 60-minute episode of middle cerebral artery occlusion in rats. One, 3, and 10 mg/kg of fasudil dose-dependently reduced blood viscosity.[13] Although the mechanism underlying the decrease of blood viscosity is not known, decrease in blood viscosity may be effective in the treatment of cerebral infarction. The multiple effects of fasudil on cerebral infarction in experimental animals suggested that it could be beneficial to human patients with cerebral ischemia.

Effect of Fasudil on Acute Cerebral Infarction in Humans

Patients and Methods

A placebo-controlled, double-blind trial of fasudil was conducted in 160 patients who had motor weakness in either or both the upper and lower extremities caused by acute cerebral infarction due to thrombosis. Patients

TABLE 68–1 Effects of Fasudil and Its Hydroxyl Metabolite (M3) on Various Protein Kinases

Kinase	K_i for Fasudil (μmol/L)	K_i for M3 (μmol/L)
Cyclic adenosine monophosphate–dependent protein kinase	**1.6**	
Cyclic guanosine monophosphate–dependent protein kinase	**1.6**	
Myosin light chain kinase	**36**	140
Protein kinase C	**3.3**	**18**
Rho kinase	0.31	0.17

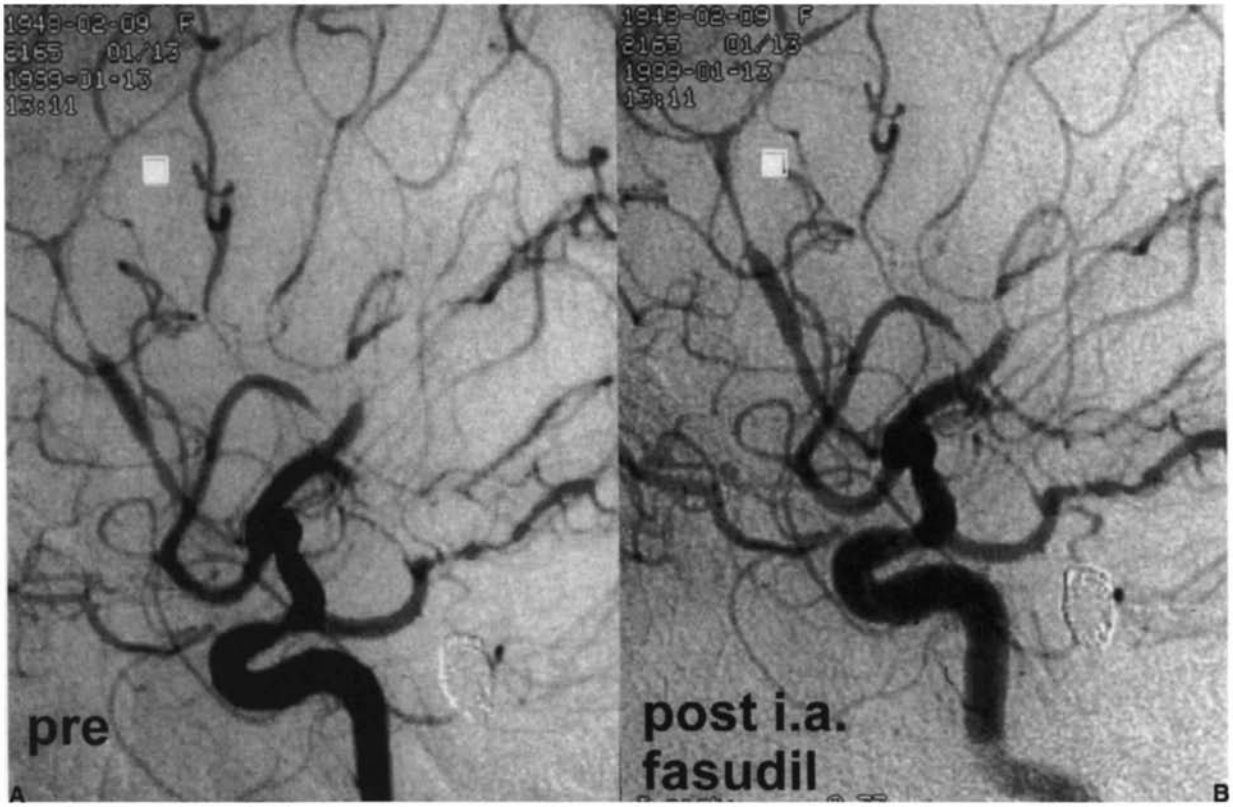


FIGURE 68–1 Photographs of lateral internal carotid artery angiograms taken before (pre, A) and after (post i.a. fasudil, B) selective intra-arterial infusion of fasudil in a patient with cerebral vasopasm after aneurysmal subarachnoid hemorrhage. There is dilation of the cerebral arteries after infusion of fasudil.

with clear consciousness or only slight disturbance (Japanese coma scale 0–3) were included in the trial. Fasudil hydrochloride (60 mg) or saline (2 mL) in 100 mL of saline was given intravenously over 60 minutes twice a day for 14 days. Patients in the placebo group were permitted to have other treatment for acute cerebral infarction including antiplatelet agents, low molecular weight dextran, and glycerol. Thrombolytics, argatroban, and ozagrel, which have been approved for acute cerebral infarction in Japan, were prohibited. Patients were examined neurologically and by CT or magnetic resonance imaging or both. Changes in motor dysfunction were examined at 14 days and final outcome was assessed at 1 month.

Results

Age, gender, and severity of infarction were similar between treated and control patients. Fasudil significantly improved both motor function and activities of daily living. Numbers of patients with improved motor function at 14 days were 30% and 19% and with worsened motor function were 3% and 18% ($p < .002$) for fasudil and placebo groups/respectively. Activities of daily living were improved in 63% and 44% and worse in 1% and 12% at 1 month follow-up in the fasudil and placebo groups, respectively ($p < .002$, Wilcoxon test). There were no difference in side effects in the two groups.

Discussion

Understanding of the mechanisms of action of fasudil on cerebral vasospasm and infarction has evolved over time from intracellular calcium antagonist to inhibitor of myosin light chain kinase, protein kinase C to inhibitor of Rho kinase. The effect of fasudil on vasospasm and infarction can be considered to be mediated by vasodilatory and anti-inflammatory mechanisms. Contraction of smooth muscle cells occurs by phosphorylation of myosin light chain at ^{19}Ser by myosin light chain kinase, which is activated by calcium-calmodulin complex. The contracted smooth muscle cells are relaxed by dephosphorylation of myosin light chain by a phosphatase. In pathological conditions such as vasospasm, Rho kinase is activated.[14,15] Activation of Rho kinase inhibits the phosphatase, leading to increased and double phosphorylation of myosin light chain at ^{19}Ser and ^{18}Thr. Fasudil inhibits this pathological double phosphorylation more specifically than the

physiological monophosphorylation,[16] which may explain how fasudil can specifically dilate spastic arteries without causing systemic hypotension. Vasodilation by fasudil may also be induced by inhibition of protein kinase C-mediated phosphorylation of the actin-calponin complex.

Fasudil increases regional cerebral blood flow in patients who have undergone surgery for ruptured intracranial aneurysms. This occurs to a similar extent on both the operated (with spasm) and nonoperated (without spasm) sides. In other words, there does not appear to be a steal phenomenon as may be seen with calcium entry blockers. The author believes that this is a very important difference between fasudil and the usual calcium entry blockers such as nimodipine or nicardipine.

It is known that polymorphonuclear leukocytes migrate into the ischemic regions of cerebral infarcts.[17] These cells produce free radicals, which are toxic to brain tissue and blood vessels. One reaction that generates free radicals after cerebral ischemia and SAH is the activation of protein kinase C, which in turn activates nicotinamide adenine diphosphate oxidase in polymorphonuclear leukocytes. Fasudil inhibits both migration of leukocytes into and production of free radicals in the ischemic region.[18]

In ischemia-induced pulmonary hypertension, there is upregulation of Rho kinase that inhibits endothelial nitric oxide synthase.[19] This can be prevented by hydroxyfasudil. This may be a mechanism by which fasudil prevents the increase in blood viscosity seen during ischemia.[13]

The usual dose of fasudil approved in Japan for prevention of vasospasm is 30 mg/100 mL of saline given over 30 minutes, three times per day for 14 days after treatment of the ruptured aneurysm by either clipping or by Guglielmi detachable coils.[5] This is a safe dose but may not be a high enough one to eradicate spasm. Generally speaking, with the preceding dosage fasudil can ameliorate ~50% of the vasospasm. Larger doses seem to be necessary depending upon the severity of SAH and the condition of the patient. If higher doses are administered, patients should be monitored closely for hypotension because fasudil increases renal blood flow and urine volume leading to dehydration and hemoconcentration, which may be detrimental to patients with vasospasm and ischemia. Meticulous control of water balance and blood pressure is important.

Conclusion

Neuroprotective effects of fasudil, which have been documented in various animal models, were proven in human cerebral vasospasm and cerebral infarction in placebo-controlled, double-blind trials. The mechanism of action of fasudil in vasospasm and infarction is considered to be due to inhibition of various protein kinases such as Rho kinase, protein kinase C, and

REFERENCES

1. Takayasu M, Suzuki Y, Shibuya M, et al. The effects of HA compound calcium antagonists on delayed cerebral vasospasm in dogs. J Neurosurg 1986;65:80–85
2. Asano T, Ikegaki I, Satoh S, et al. Mechanism of action of a novel antivasospasm drug, HA1077. J Pharmacol Exp Ther 1987;241: 1033–1040
3. Shibuya M, Asano T, Sasaki Y. Effect of fasudil HC1, a protein kinase inhibitor, on cerebral vasospasm. Acta Neurochir Suppl 2001;77:201–204
4. Shimokawa H, Seto M, Katsumata N, et al. Rho-kinase-mediated pathway induces enhanced myosin light chain phosphorylation in a swine model of coronary artery spasm. Cardiovasc Res 1999; 43:1029–1039
5. Shibuya M, Suzuki Y, Sugita K, et al. Effect of AT877 on cerebral vasospasm after aneurysmal subarachnoid hemorrhage: results of a prospective placebo-controlled double blind trial. J Neurosurg 1992;76:571–577
6. Fisher CM, Kistler JP, Davis JM. Relation of cerebral vasospasm to subarachnoid hemorrhage visualized by computerized tomographic scanning. Neurosurgery 1980;6:1–9
7. Shibuya M, Oosuka K, Suzuki Y, et al. Clinical and angiographic effect of fasudil HC1 (AT877) on cerebral vasospasm. In: Dorsch N, ed. Cerebral Vasospasm VI, Proceedings of the VIth International Conference on Cerebral Vasospasm. Leichhardt NSW, Australia: Oslington Consulting Pty Ltd; 1999:261–263
8. Tachibana E, Harada T, Shibuya M, et al. Intra-arterial infusion of fasudil hydrochloride for treating vasospasm following subarachnoid haemorrhage. Acta Neurochir (Wien) 1999;141:13–19
9. Satoh S, Ikegaki I, Suzuki Y, et al. Neuroprotective properties of a protein kinase inhibitor against ischaemia-induced neuronal damage in rats and gerbils. Br J Pharmacol 1996;118:1592–1596
10. Tsuchiya M, Sako K. The effect of HA1077, a novel protein kinase inhibitor, on reduction of cerebral blood flow and glucose metabolism following acute and/or chronic bilateral carotid artery ligation in Wistar rats. Exp Brain Res 1993;97:233–238
11. Toshima Y, Satoh S, Ikegaki I, et al. A new model of cerebral microthrombosis in rats and the neuroprotective effect of a rho-kinase inhibitor. Stroke 2000;31:2245–2250
12. Satoh S, Kobayashi T, Hitomi A, et al. Inhibition of neutrophil migration by a protein kinase inhibitor for the treatment of ischemic brain infarction. Jpn J Pharmacol 1999;80:41–48
13. Hitomi A, Satoh S, Ikegaki I, et al. Hemorheological abnormalities in experimental cerebral ischemia and effects of protein kinase inhibitor on blood fluidity. Life Sci 2000;67:1929–1939
14. Katsumata N, Shimokawa H, Seto M, et al. Enhanced myosin light chain phosphorylation as a central mechanism for coronary artery spasm in a swine model with interleukin 1-β. Circulation 1997;96:4357–4363
15. Miyagi Y, Carpenter RC, Meguro T, et al. Upregulation of rho A and rho kinase messenger RNAs in the basilar artery of a rat model of subarachnoid hemorrhage. J Neurosurg 2000;93: 471–476
16. Seto M, Sasaki Y. Intimal hyperplasia enhances myosin phosphorylation in rabbit carotid artery. Exp Mol Pathol 1993;58:1–13
17. Akopov SE, Simonian NA, Grigorian GS. Dynamics of polymorphonuclear leukocyte accumulation in acute cerebral infarction and their correlation with brain tissue damage. Stroke 1996;27:1739–1743
18. Satoh S, Yamamoto Y, Toshima Y, et al. Fasudil, a protein kinase inhibitor, prevents the development of endothelial injury and neutrophil infiltration in a two-haemorrhage canine subarachnoid model. J Clin Neurosci 1999;6:394–399
19. Takemoto M, Sun J, Hiroki J, et al. Rho-kinase mediates hypoxia-induced downregulation of endothelial nitric oxide synthase. Circulation 2002;106:57–62

69

Fasudil Hydrochloride for Vasospasm After Early Surgery for Good Grade Patients with Subarachnoid Hemorrhage

YOSHITSUGU NUKI, M.D., PH.D., SHINICHI NAKANO, M.D., PH.D., NAOKI ASAMI, M.D., TAKUZO MORIYAMA, M.D., PH.D., HISAO UEHARA, M.D., PH.D., HIROKAZU KAWANO, M.D., PH.D., TOMOKAZU GOYA, M.D., PH.D., SHINICHIRO WAKISAKA, M.D., PH.D.

Abstract

We retrospectively evaluated the efficacy of fasudil hydrochloride in prevention of symptomatic cerebral vasospasm after aneurysmal subarachnoid hemorrhage (SAH). Surgical clipping of ruptured aneurysms was performed within 72 hours of SAH in 248 patients with Hunt and Kosnik grades 1 to 3 between 1993 and 1999. The degree and extent of SAH was assessed with preoperative cranial computed tomography using Fisher's classification. In the first 3 years, 123 patients were treated with modified hemodynamic therapy (hypertension, hypervolemia, and hemodilution) alone for the prevention of vasospasm. These patients were used as historical controls in a comparison with a cohort of 125 patients that were treated in the past 4 years. These patients were treated with a combination of the above hemodynamic therapy and intravenous fasudil hydrochloride (90 mg/day for 14 days). Clinical outcome was assessed at 3 months using the Glasgow outcome scale. Statistical analyses were performed by χ-square statistics. Odds ratios and their 95% confidence intervals were calculated by logistic regression analysis. There were no significant differences in age, gender, clinical, and computed tomographic (CT) grades and location of ruptured aneurysms between the two groups. The cohort treated with fasudil had a significantly lower incidence of symptomatic vasospasm (28 vs 7%, $p < .0001$, χ-square test: odds ratio, 0.20, 95% confidence interval, 0.09–0.45). The rate of good recovery was also higher in the fasudil-treated patients (58 vs 71%, $p = .027$, χ-square test: odds ratio, 1.8, 95% confidence interval, 1.1–3.1). Although definitive conclusions regarding the efficacy of fasudil cannot be drawn in this nonrandomized study, the results suggest that fasudil may reduce the incidence of symptomatic cerebral vasospasm in patients with Hunt and Kosnik grade 1 to 3 SAH, resulting in better clinical outcome.

Cerebral vasospasm is a serious complication of subarachnoid hemorrhage (SAH) and contributes to death and neurological deficits. In patients with poor grade SAH, the initial damage rather than vasospasm may be a major cause of poor clinical outcome. In those who are in good clinical grades after SAH, however, vasospasm may be a more important cause of poor clinical outcome than the initial brain damage. Fasudil

hydrochloride, a chemical compound synthesized in Japan, is a new type of Ca^{2+} antagonist that directly affects intracellular Ca^{2+} ions. The mechanism of action is different from traditional Ca^{2+} antagonists such as nimodipine that simply block the influx of Ca^{2+} ions. Fasudil has been developed as a unique cerebral vasodilator and there are numerous reports describing its effects. Fasudil inhibits several protein kinases involved in smooth muscle contraction including myosin light chain kinase, protein kinase C, and Rho kinase.[1–3] Although inhibition of myosin light chain kinase might inhibit contraction in a broad range of muscle tissues, inhibition of protein kinase C might specifically affect the cerebral vessels during vasospasm because it has been suggested that some of the mechanism of vasospasm depends on activation of protein kinase C.[1,2] Rho kinase may be an even more important kinase in vasospasm.[4,5] The efficacy of fasudil for prevention of vasospasm has been demonstrated in several experimental and clinical studies.

The purpose of the present study was to confirm the favorable effects of fasudil hydrochloride on symptomatic vasospasm and clinical outcome in patients with Hunt and Kosnik grade 1 to 3 SAH.[6]

Subjects and Methods

A total of 285 patients with Hunt and Kosnik grade 1 to 3 SAH were admitted to our hospital between 1993 and 1999. The 248 patients who underwent surgery to clip the ruptured aneurysm within 72 hours of SAH were included in this study. The degree and extent of SAH was assessed with preoperative computed tomography (CT) using Fisher's classification.[7] During the first 3 years, 123 patients were treated with modified hemodynamic therapy (hypertension, hypervolemia, and hemodilution) only for prevention of vasospasm and were used as controls. The subsequent 125 patients were accrued over the past 4 years and were treated with a combination of the preceding hemodynamic therapy plus intravenous fasudil hydrochloride (90 mg/day for 14 days, Table 69–1). Symptomatic vasospasm was diagnosed when there was onset of new symptoms that were not found immediately after operation, such as headache, disorientation, aphasia, and hemiparesis. When such symptoms appeared, cerebral angiography or CT angiography was performed immediately to determine whether the symptoms were caused by vasospasm. Patients were treated first with rapid intravenous infusion of low molecular weight dextran (500 mL in 2 hours) after confirming that there were no low-density areas related to vasospasm on the CT scan. If necessary, percutaneous transluminal angioplasty, intra-arterial administration of low-dose papaverine hydrochloride, or both were performed. In patients with asymptomatic vasospasm and those with no vasospasm, postoperative catheter or three-dimensional CT angiography were performed within 15 days of SAH.

Clinical outcome was assessed at 3 months after onset using the Glasgow outcome scale.[8] Statistical analyses were performed by χ-square analysis. Odds ratios and corresponding 95% confidence intervals were calculated by logistic regression analysis. A $p < .05$ was considered statistically significant. A single neurosurgeon (Y.N.) evaluated all of the patients at baseline and reviewed medical records, neuroimaging, the diagnosis of vasospasm, and the Glasgow outcome score at 3 months from SAH.

Results

There were no significant difference in age, sex, clinical, and CT grades and location of ruptured aneurysms between the two groups at baseline (Table 69–2). Table 69–3 shows the incidence of symptomatic vasospasm and the clinical outcome

TABLE 69–1 Clinical Management of Patients with Subarachnoid Hemorrhage from Ruptured Cerebral Aneurysms

Cranial CT on arrival and if necessary a three-dimensional CT angiogram
Cerebral angiography more than 6 hours after onset of SAH
Surgery for aneurysm clipping within 72 hours of SAH
Subarachnoid clot removal by perioperative irrigation
Postoperative continuous clot removal by cisternal or spinal drainage
Early nutritional supplementation by hyperalimentation
Modified hemodynamic therapy (normotension or moderate hypertension, hypervolemia, and hemodilution)
Intravenous administration of fasudil hydrochloride (30 mg of fasudil hydrochloride was infused over 30 minutes 3 times a day for 14 days)
Percutaneous transluminal angioplasty if necessary
Intra-arterial administrations of low-dose papaverine hydrochloride if necessary
Postoperative angiography and/or three-dimensional CT angiography within 15 days of the operation

CT, computed tomography; SAH, subarachnoid hemorrhage.

TABLE 69–2 Characteristics of 248 Patients with Subarachnoid Hemorrhage*

	1993–1995, no fasudil (n = 123)	1996–1999, fasudil (n = 125)	χ-square test
Age (years)	**60** ±12	**60** ±13	Not significant
Sex			Not significant
Male	**51 (42%)**	**47 (38%)**	
Female	**72 (59%)**	**78 (62%)**	
Hunt and Kosnik grade			Not significant
1	**13 (11%)**	**14 (11%)**	
2	**52 (42%)**	**59 (47%)**	
3	**58 (47%)**	**52 (42%)**	
Fisher's grade on CT scan			Not significant
1	**4(3%)**	**2 (2%)**	
2	**37 (30%)**	**29 (23%)**	
3	**62 (50%)**	**73 (58%)**	
4	**20 (16%)**	**21 (17%)**	
Location of aneurysms			Not significant
ICA	**35 (28%)**	**35 (28%)**	
MCA	**37 (30%)**	**28 (22%)**	
ACA	**48 (39%)**	**58 (47%)**	
VB	**3 (2%)**	**4 (3%)**	

*Values are means ± standard deviations. ACA, anterior cerebral artery; CT, computed tomography; ICA, internal carotid artery; MCA, middle cerebral artery; VB, vertebrobasilar.

for the two groups. Patients treated with fasudil had had a significantly lower incidence of symptomatic vasospasm (28 vs 7%, $p < .0001$, χ-square test: odds ratio, 0.20, 95% confidence interval, 0.09–0.45). The rate of good recovery was also higher in the fasudil-treated patients (58 vs 71%, $p = .027$, χ-square test: odds ratio, 1.8, 95% confidence interval, 1.1–3.1). Vasospasm and mortality also were significantly less frequent in the cohort treated with fasudil ($p < .01$, χ-square test).

Discussion

This study suggests that the use of fasudil hydrochloride may decrease symptomatic vasospasm and improve clinical outcome in patients with aneurysmal

TABLE 69–3 Overall Results and Symptomatic Cerebral Vasospasm in Two Groups of Patients

	1993–1995, No fasudil) (n = 123)		1996–1999, fasudil (n = 125)	
Glasgow Outcome	N(%)	**Patients with Symptomatic Vasospasm (%)**	N(%)	**Patients with Symptomatic Vasospasm** (%)
Good recovery	**71 (58%)**	**6 (5%)**	**89 (72%)***	2 (2%)
Moderate disability	**26 (21%)**	**15 (12%)**	**19 (15%)**	4 (3%)§
Severe disability	**5 (4%)**	**1 (1%)**	**9 (7%)**	1 (1%)
Vegetative state	**4 (3%)**	**3 (2%)**	**3 (2%)**	1 **(1%)**
Death	**17 (14%)**	**9 (7%)**	5 (4%)§§	1 (1%)+
Total	123 (100%)	**34 (28%)**	**125 (100%)**	9 (7%)++

*$p < .05$ compared with group not treated with fasudil.
§/$p = .008$ compared with group not treated with fasudil.
§§$p = .007$ compared with group not treated with fasudil.
†$p = .009$ compared with group not treated with fasudil.
††$p < .0001$ compared with group not treated with fasudil.

SAH. The limitations of the study include the retrospective nature, use of historical controls, and accrual of patients over 7 years, which leads to the concern that improvement in general management of the patients could contribute to the differences. Therefore, definitive statements about the benefits of fasudil cannot be made. The incidence of symptomatic vasospasm in our first group of patients was 28%, which is quite similar to the results of other studies in the late 1990s that reported the incidence of symptomatic vasospasm to be in the vicinity of 30%.[1] Compared with these results, the incidence of symptomatic vasospasm in our subsequent group of patients that were treated with fasudil was 7%, which is considerably lower, suggesting that fasudil reduces symptomatic vasospasm. The present study also demonstrated that the significant decrease in symptomatic vasospasm may result in a significant increase in the rate of good recovery and decrease in the rate of death when the patient population was restricted to those with Hunt and Kosnik grade 1 to 3 SAH.

In conclusion, our therapeutic protocol using fasudil hydrochloride after early clipping surgery may be effective for preventing symptomatic vasospasm and improving clinical outcome.

REFERENCES

1. Shibuya M, Asano T, Sasaki Y. Effects of Fasudil HCl, a protein kinase inhibitor, on cerebral vasospasm. Acta Neurochir Suppl 2001;77:201–204
2. Seto M, Sasaki Y, Hidaka H, Sasaki Y. Effects of HA1077, a protein kinase inhibitor, on myosin phosphorylation and tension in smooth muscle. Eur J Pharmacol 1991;195:267–272
3. Asano T, Suzuki Y, Tsuchiya M, et al. Vasodilator actions of HA1077 in vitro and in vivo putatively mediated by the inhibition of protein kinase. Br J Pharmacol 1989;98:1091–1100
4. Nagumo H, Sasaki Y, Ono Y, et al. Rho kinase inhibitor HA-1077 prevents Rho-mediated myosin phosphatase inhibition in smooth muscle cells. Am J Physiol 2000;278:C57–C65
5. Uehata M, Ishizaki T, Satoh H, et al. Calcium sensitization of smooth muscle mediated by a Rho-associated protein kinase in hypertension. Nature 1997;389:990–994
6. Hunt WE, Kosnik EJ. Timing and perioperative care in intracranial aneurysm surgery. Clin Neurosurg 1974;21:79–89
7. Fisher CM, Kistler JP, Davis JM. Relation of cerebral vasospasm to subarachnoid hemorrhage visualized by computerized tomographic scanning. Neurosurgery 1980;6:1–9
8. Jennett B, Bond M. Assessment of outcome after severe brain damage: a practical scale. Lancet 1975;1:480–484

70

Analysis of Patients with Cerebral Infarction Caused by Vasospasm after Intra-Arterial Administration of Fasudil Hydrochloride

SATOSHI IWABUCHI, M.D., TETSUYA YOKOUCHI, M.D., KAZUYA AOKI, M.D., HITOSHI KIMURA, M.D., MORITO HAYASHI, M.D., *Go* NAKAGAWA, M.D., HIRONORI FURUKAWA, M.D., MORIKAZU UEDA, M.D., HIROTSUGU SAMEJIMA, M.D.

Abstract

We have reported that intra-arterial administration of fasudil hydrochloride to patients with vasospasm can result in angiographic and clinical improvement. Despite these positive effects, we still encounter patients who develop symptomatic vasospasm. This study analyzed prognostic factors predicting development of low density areas due to vasospasm on cranial computed tomographic (CT) scans. A population of 62 patients undergoing surgery for ruptured aneurysms were studied with angiography 7 to 9 days after subarachnoid hemorrhage (SAH). Thirty-two patients had vasospasm and had intra-arterial infusion of fasudil. Eight patients developed low density areas due to vasospasm on CT scan. Six of these patients had undergone surgical clipping (14% of 43 surgically treated patients) and two had been treated endovascularly (11% of 19, not statistically significantly different). Seven patients with symptomatic vasospasm developed fever above 38°C, and one had elevated platelet count > 450,000/μL. The clinical outcomes of these eight patients were good recovery in three, moderate disability in three, severe disability in one, and vegetative state in one. Intra-arterial fasudil resulted in better outcome in patients with SAH. It was also found that the management of body temperature was especially important for prevention of symptomatic vasospasm.

Fasudil hydrochloride is widely administered in Japan to prevent cerebral vasospasm in patients following subarachnoid hemorrhage (SAH). We have reported that intra-arterial administration of fasudil hydrochloride results in vasodilation, improvement of circulatory dynamics, and better outcomes in patients with SAH. Despite these positive results, we still encounter patients who develop symptomatic vasospasm. This study analyzed prognostic factors predicting development of low density areas due to vasospasm on cranial computed tomographic (CT) scans.

Clinical Materials and Methods

Between September 1998 and December 2002, fasudil hydrochloride was administered intravenously to

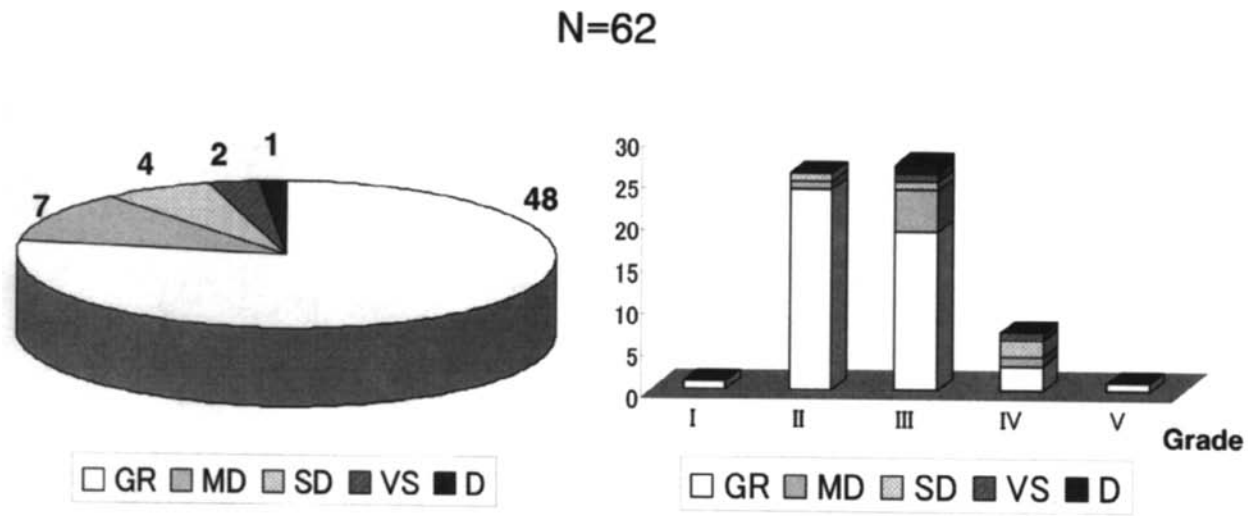


FIGURE 70–1 Pie chart (left) showing overall recovery of 62 patients with subarachnoid hemorrhage (SAH), based on the Glasgow outcome scale. GR, good recovery; MD, moderate disability; SD, severe disability; VS, vegetative state; D, death. Bar graph (right) shows recovery on the Glasgow outcome scale stratified by the preoperative neurological grade based on the Hunt and Kosnik grade.

62 patients who had undergone radical treatment for ruptured aneurysms within 72 hours of SAH. Follow-up angiography was performed 7 to 9 days after SAH to determine the presence or absence of angiographic vasospasm. When vasospasm was demonstrated on angiography, 15 mg of fasudil hydrochloride was infused into the internal carotid or vertebral artery over 10 minutes. Infusions were given into each affected vascular territory. Thirty-three patients had angiographic vasospasm and fasudil hydrochloride was

TABLE 70–1 Summary of Eight Patients with SAH Treated with Intra-arterial Fasudil and Showing Ischemic Changes on Computed Tomographic Scan

Age	Sex	Hunt and Kosnik Grade	CT Fisher Grade	Aneurysm Site	Vascular Territory	Day of Symptomatic Vasospasm	Day CT Scan Showed Low-Density Area	Glasgow Outcome Score
82	Female	3	3	Right posterior communicating	Right anterior cerebral	11	11	Good recovery
51	Female	3	3 + intracerebral hemorrhage	Anterior communicating	Left anterior cerebral, middle cerebral	10	12	Moderate disability
63	Female	2	3	Anterior communicating	Right middle cerebral	11	11	Severe disability
50	Male	3	3	Left posterior communicating	Bilateral anterior cerebral	6	8	Good recovery
58	Male	3	3	Right posterior communicating	Right middle cerebral	11	13	Moderate disability
79	Female	4	3	Left middle cerebral	Left middle cerebral	—	14	Severe disability
57	Female	4	3	Left middle cerebral	Left middle cerebral	11	12	Moderate disability
63	Male	2	3 + intracerebral hemorrhage	Anterior communicating	Bilateral anterior cerebral		9	Good recovery

administered intra-arterially to each patient. Medical records, radiographic studies, intensive care parameters, and clinical outcome at discharge were reviewed for patients found to have low-density areas due to vasospasm on CT scans.

Results

The 62 patients consisted of 20 men and 42 women ranging in age from 20 to 82 years (mean age of 57 years). The clinical outcomes using the Glasgow outcome scale were good recovery in 48, moderate disability in seven, severe disability in four, vegetative state in two, and death in one, respectively (Fig. 70–1).[1] Outcome according to the preoperative Hunt and Kosnik neurological grade is also given.[2] The single fatality was the result of disseminated intravascular coagulation caused by a drug reaction. Six of 43 patients who underwent surgical clipping (14%) and 2 of 19 patients who underwent endovascular treatment (11%) had low-density areas on CT scan. The incidence of low-density areas on CT scans was lower in the endovascular treatment group, but the difference was not statistically significant. Eight patients (13%) developed low-density areas on CT scans due to vasospasm. Seven patients with symptomatic vasospasm developed fever above 38°C, and one patient had a platelet count > 450,000/ML.

The clinical outcomes of these eight patients were good recovery in three, moderate disability in three, severe disability in one, and vegetative state in one. CT scans of the three patients who made good recoveries revealed ischemic changes in the anterior cerebral artery territory but not the middle cerebral artery territory (Table 70–1).[3] Systemic blood pressure was not decreased after intra-arterial administration of fasudil hydrochloride in any patient. Patients were also monitored for the possibility of other adverse effects, such as autonomic symptoms, convulsions, and disturbance of consciousness, which may be caused by intra-arterial administration of papaverine. None of

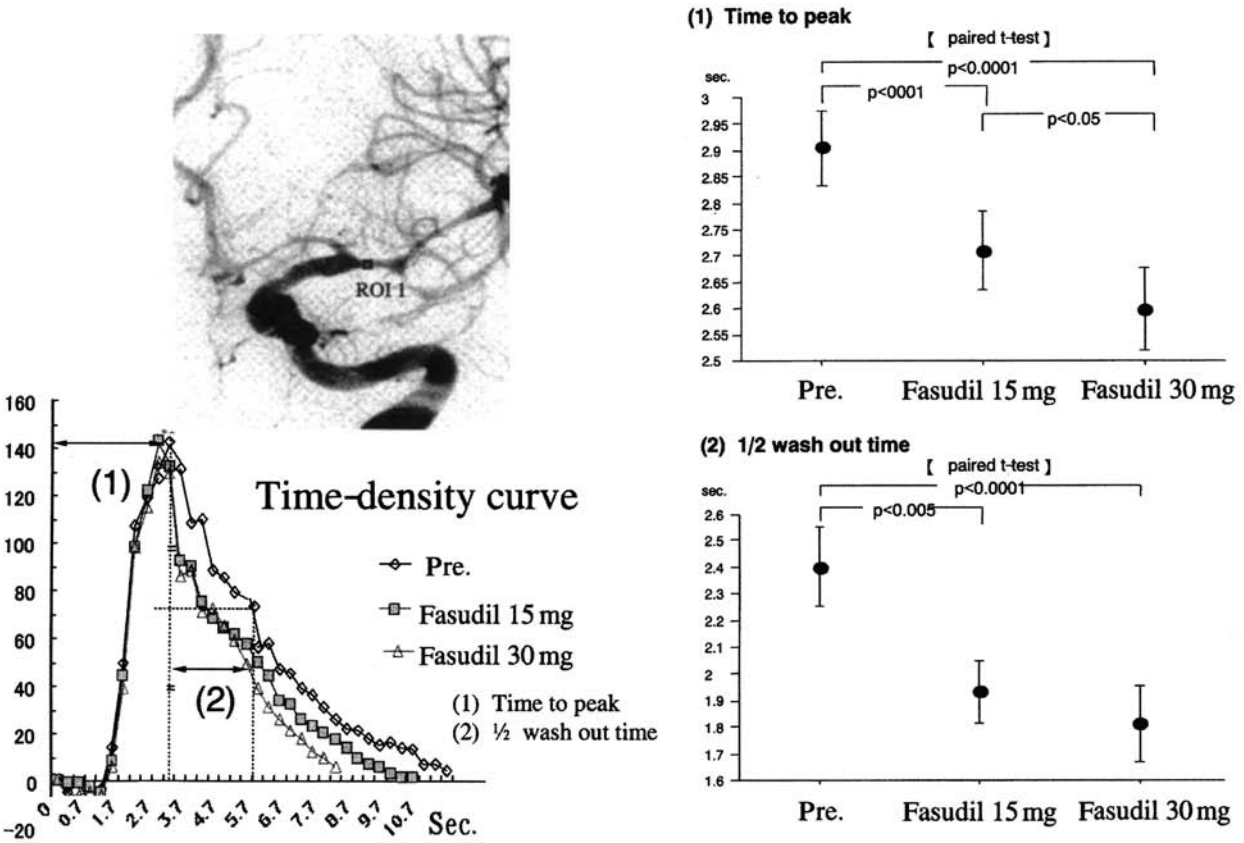


FIGURE 70–2 Upper left: photograph of angiogram of patient with vasospasm showing selection of a region of interest (ROI) for measurement of time-density curves. Lower left: graph showing time density curves in a patient before and after infusion of 15 and 30 mg fasudil intra-arterially and the method of measurement of time to peak and half washout time. Upper right: graph showing results of time to peak before and after 15 and 30 mg fasudil. Lower right: graph showing results of half washout time before and after 15 and 30 mg fasudil.

these adverse symptoms was encountered in association with fasudil hydrochloride.

Discussion

Fasudil hydrochloride is a Ca^{2+} antagonist that has a different mechanism of action from Ca^{2+} entry blockers such as nimodipine or nicardipine. Fasudil hydrochloride inhibits myosin light chain kinase, which is an essential component in smooth-muscle contraction. Fasudil hydrochloride also inhibits Rho-associated protein kinase, which contributes to vascular smooth muscle contraction by inactivating myosin phosphatase. Fasudil hydrochloride is now widely used to prevent vasospasm in patients with subarachnoid hemorrhage in Japan. We have reported that intra-arterial administration of fasudil hydrochloride causes diffuse dilation of proximal arteries, as well as of distal vessels, resulting in an improvement of cerebral circulatory dynamics. We examined the effect of fasudil on circulatory dynamics during cerebral angiography Both the time to peak and time to half washout were reduced on the time-density curve after intra-arterial administration of fasudil hydrochloride (Fig. 70–2). These times were reduced at proximal and distal points in the circulation as well as at the transverse sinus. These results suggest that intra-arterial administration of fasudil hydrochloride may improve capillary circulation. Patients treated with intra-arterial fasudil had better outcome at discharge and a lower overall mortality rate.

We encountered eight patients (13%) who developed cerebral infarction due to vasospasm in the present series. Three of these patients went on to make good recoveries based on the Glasgow outcome score. The CT scans of all three of these patients revealed ischemic changes in the territory of the anterior cerebral but not the middle cerebral artery. We believe that if a single intra-arterial administration of fasudil hydrochloride is insufficient, repeated intra-arterial administration of fasudil or addition of balloon angioplasty to preserve flow in the middle cerebral artery territory may lead to a better prognosis.

REFERENCES

1. Jennett B, Bond M. Assessment of outcome after severe brain damage: a practical scale. Lancet 1975;1:480–484
2. Hunt WE, Kosnik EJ. Timing and perioperative care in intracranial aneurysm surgery. Clin Neurosurg 1974;21:79–89
3. Fisher CM, Kistler JP, Davis JM. Relation of cerebral vasospasm to subarachnoid hemorrhage visualized by computerized tomographic scanning. Neurosurgery 1980;6:1–9

71

Therapeutic Strategies for Cerebral Vasospasm after SAH: Efficacy of a Combination of Intraventricular Urokinase and Intra-Arterial Fasudil

SHIGEKI ONO, M.D., TOMOHITO HISHIKAWA, M.D., MITSUHISA NISHIGUCHI, M.D., SHINSAKU NISHIO, M.D., KOJI TOKUNAGA, M.D., KENJI SUGIU, M.D., ISAO DATE, M.D.

Abstract

Cerebral vasospasm after subarachnoid hemorrhage (SAH) is still an important cause of death and disability despite the development of several therapeutic strategies. Injection of urokinase into the subarachnoid space may speed the clearance of subarachnoid clot and thereby reduce the severity of vasospasm although the route of administration and dosage remain open to question. We have used urokinase in combination with a new type of vasodilatory agent, fasudil hydrochloride, which is considered to act via inhibition of Rho kinase, for treatment of vasospasm in patients with aneurysmal SAH. Patients who presented with SAH (Fisher group 2–4) were treated with injection of boluses of urokinase into the lateral ventricles and with intra-arterial fasudil hydrochloride injection if vasospasm occurred (n = 11). Lumbar and ventricular drains were inserted in these patients. Results were compared with a group of historical controls that were treated with lumbar, cisternal, or ventricular drainage (n = 13). Hemodynamic therapy was conducted in all patients if symptomatic vasospasm developed. The frequency of angiographic and symptomatic vasospasm was assessed and the outcome was analyzed in each group. Angiographic vasospasm was seen in 91% of treated patients and 53% of controls and symptomatic vasospasm in 64 and 46%, respectively Good outcome (Glasgow outcome score of good recovery or moderate disability) occurred in 100% of treated and 69% of control patients. The combination of intraventricular bolus injection of urokinase and intra-arterial fasudil injection was effective at preventing symptomatic vasospasm and improving outcome.

Cerebral vasospasm remains an important cause of morbidity and mortality in patients with aneurysmal subarachnoid hemorrhage (SAH). Many therapeutic strategies to prevent or treat vasospasm have been reported,[1,2] but there still are no absolutely effective therapies. It is known that the amount of blood in the subarachnoid space after SAH correlates strongly with the degree of vasospasm.[3–5] Injection of thrombolytic drugs such as urokinase into the subarachnoid space after SAH may promote more rapid washout of subarachnoid blood clots and thereby reduce cerebral vasospasm. It has not been clear which route of

administration is most effective or what dose is required. This study assessed one method of administration of urokinase. In addition, we have tested superselective intra-arterial injections of a new type of vasodilatory agent, fasudil hydrochloride, which is considered to act as a Rho kinase inhibitor, for the treatment of vasospasm by intra-arterial superselective injection.

Methods

The combination of urokinase bolus injection into the lateral ventricle and intra-arterial fasudil injection has been used in 11 patients with SAH since January 2002. Thirteen patients who were managed before this time were selected as controls. All patients had Fisher grade 2 to 4 SAH on cranial computed tomography.[6] Patients managed before January 2002 did not receive intraventricalar urokinase or intra-arterial fasudil. In the treated patients bolus injection of urokinase into the lateral ventricle or intra-arterial fasudil hydrochloride injection was performed when cerebral vasospasm occurred. Angiography was performed routinely 7 days after SAH. Fasudil hydrochloride, 15 to 30 mg, was injected through a microcatheter inserted into the sphenoidal segment of the middle cerebral or precommunicating segment of the anterior cerebral artery over 20 minutes. The injection was repeated if dilation was not complete. A lumbar, cisternal, or ventricular drain was inserted in the historical controls whereas the treated patients had a lumbar or cisternal plus ventricular drain placed. When vasospasm occurred, hypertension and hypervolemic therapy was instituted in all patients. The frequency of angiographic and symptomatic vasospasm was assessed, and the outcome was analyzed in each group.

Data are given as means ± standard deviations with significance as $p < .05$. Comparisons between groups were by analysis of χ^2 test with Yate's correction for 2 × 2 tables.

Results

All patients underwent clipping of the ruptured aneurysm within 72 hours of SAH. Patient age, sex, Hunt and Kosnik grade,[7] and aneurysm location were similar between groups (Table 71–1). Intravascular treatment was performed in eight patients in the treated group. In addition, two of these patients had percutaneous angioplasty for vasospastic arteries. Angiographic vasospasm was seen in 53% (*n = 7)* of control and 91% (*n* = 10) of treated patients, and symptomatic vasospasm was observed in 46% (*n* = 6) and 64% (*n = 7)* of these groups, respectively (Fig. 71–1). The outcome was assessed at discharge from the hospital. Sixty-nine percent of the controls (*n* = 9) and 100% of the treated patients (*n* = 11) made favorable outcomes as judged by the Glasgow outcome scale (good recovery or moderate disability, Figs. 71–2 and 71–3).[8] There were no significance differences between groups in the frequency of angiographic and symptomatic vasospasm. Outcome was, however, better in treated than in control patients (see Figs. 71–2 and 71–3, $p < .05$). There were no deaths in either group.

TABLE 71–1 Characteristics of Patients in Treated and Control Groups*

Characteristic	Controls *in* = 13)	Urokinase/Fasudil (*n* = 11)
Age (years)	**61** ±15	**61 ±9**
Male/female	**5/8**	**6/5**
Hunt and Kosnik grade	**2.2 ± 1.2**	**2.5 ± 1.0**
Anterior/posterior circulation	10/3	11/0

* Values are means ± standard deviations.

Discussion

Although the precise mechanism of vasospasm is still unknown, it is generally accepted that the amount of blood clot in the subarachnoid space after SAH correlates with the degree of vasospasm.[3–5] Kodama et al reported that cisternal irrigation with urokinase solution after the surgical treatment of ruptured intracranial aneurysms produces good results with a low incidence of vasospasm.[9] Urokinase is one of several fibrinolytic agents that have been used for this purpose. In addition, there are numerous injection routes and doses that have been used.[10] In this report, we

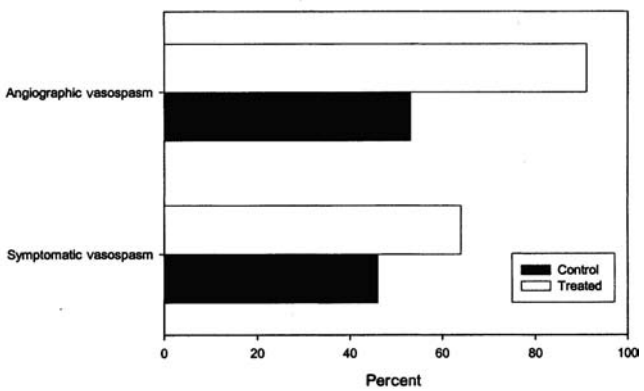


FIGURE 71–1 Incidence of symptomatic and angiographic vasospasm in patients treated with intraventricular urokinase and intra-arterial fasudil and in patients in the control group. There were no significant differences between groups.

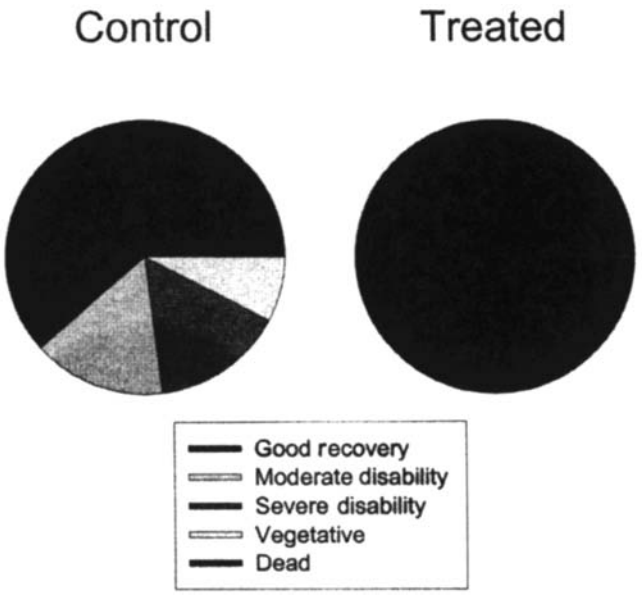


FIGURE 71–2 Outcome based on Glasgow outcome score at discharge for patients treated with intraventricular urokinase and intra-arterial fasudil and patients in the control group.

adopted the intraventricular bolus injection method to deliver urokinase because it is easy to perform, a ventricular drain is useful for controlling intracranial pressure, and it is possible to use this method for clot lysis in cases where craniotomy is not performed, such as those patients who undergo coil embolization of the ruptured aneurysm.

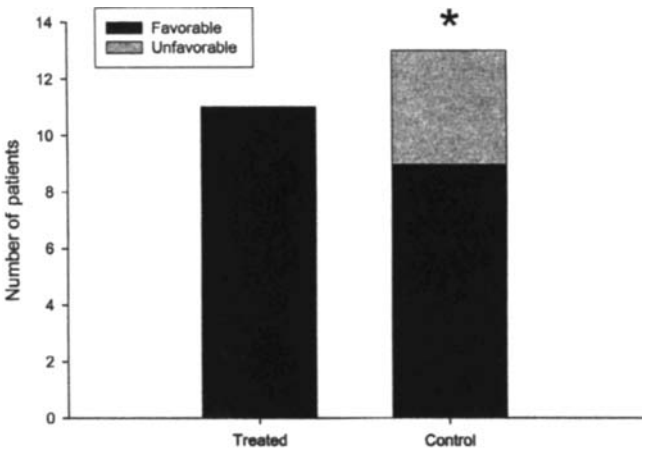


FIGURE 71–3 Number of patients with favorable (good recovery and moderate disability on the Glasgow outcome scale) and unfavorable outcome (severe disability, vegetative, and dead) for the two groups. Groups are patients treated with intraventricular urokinase and intra-arterial fasudil and patients in the control group. There were significantly more favorable outcomes in the treated group ($p < .05$).

This method did not, however, significantly reduce angiographic or symptomatic vasospasm compared with historical controls. We noted that in some cases in this study, clots disappeared quickly after urokinase injection but in other cases, clot clearance was less rapid. Our results suggest that it may be difficult to remove subarachnoid clots completely using intraventricular urokinase. Alternate injection routes or frequency of administration may be needed.

Fasudil hydrochloride is widely used for prevention of vasospasm in patients with SAH in Japan. It generally is administered intravenously. Fasudil is thought to prevent the abnormal dual phosphorylation of myosin light chain kinase that occurs in vasospasm through the inhibition of Rho kinase. Some institutions in Japan have used this agent for intra-arterial treatment of vasospasm in a similar fashion to what has been described for papaverine.[11] We used selective intra-arterial injections of fasudil hydrochloride for the treatment of vasospasm and suggest that this effectively reversed vasospasm. The effect may be achieved because higher concentrations can be administered intra-arterially than could be given intravenously without causing hypotension or disordered renal function. We did not observe these side effects in patients receiving intra-arterial fasudil in this study. In our experience, intra-arterial injection of fasudil hydrochloride is more effective on more distal, peripheral vasospasm. Therefore, the combination of percutaneous transluminal angioplasty with selective intra-arterial injection of fasudil may be a useful therapy for vasospasm. Further studies are being conducted in our institution to clarify the effectiveness of this therapy and investigate the most effective combination of these treatments.

Conclusion

Multimodality therapy using intraventricular injection of urokinase and intra-arterial injection of fasudil hydrochloride improved outcome in a small series of patients with aneurysmal SAH compared with historical controls. However, there was no significant effect on angiographic or symptomatic vasospasm. One explanation of this paradox is that prevention of vasospasm with vasodilation therapy needs to address both large proximal and smaller peripheral cerebral arteries to reduce symptomatic vasospasm. Study of more patients who are treated by this combination therapy is required.

REFERENCES

1. Treggiari-Venzi MM, Suter PM, Romand JA. Review of medical prevention of vasospasm after aneurysmal subarachnoid hemorrhage: a problem of neurointensive care. Neurosurgery 2001; 48:249–261

2. Macdonald RL, Weir B. Cerebral Vasospasm. San Diego: Academic; 2001:1–518

3. Weir B, Macdonald RL, Stoodley M. Etiology of cerebral vasospasm. Acta Neurochir Suppl (Wien) 1999;72:27–46

4. Kassell NF, Sasaki T, Colohan AR, Nazar G. Cerebral vasospasm following aneurysmal subarachnoid hemorrhage. Stroke 1985; 16:562–572

5. Friedman JA, Goerss SJ, Meyer FB, et al. Volumetric quantification of Fisher grade 3 aneurysmal subarachnoid hemorrhage: a novel method to predict symptomatic vasospasm on admission computerized tomography scans. J Neurosurg 2002; 97:401–407

6. Fisher CM, Kistler JP, Davis JM. Relation of cerebral vasospasm to subarachnoid hemorrhage visualized by computerized tomographic scanning. Neurosurgery 1980;6:1–9

7. Hunt WE, Kosnik EJ. Timing and perioperative care in intracranial aneurysm surgery. Clin Neurosurg 1974;21:79–89

8. Jennett B, Bond M. Assessment of outcome after severe brain damage: a practical scale. Lancet 1975;1:480–484

9. Kodama N, Sasaki T, Kawakami M, Sato M, Asari J. Cisternal irrigation therapy with urokinase and ascorbic acid for prevention of vasospasm after aneurysmal subarachnoid hemorrhage: outcome in 217 patients. Surg Neurol 2000;53:110–117

10. Moriyama E, Matsumoto Y, Meguro T, et al. Combined cisternal drainage and intrathecal urokinase injection therapy for prevention of vasospasm in patients with aneurysmal subarachnoid hemorrhage. Neurol Med Chir (Tokyo) 1995;35:732–736

11. Masaoka H, Takasato Y, Nojiri T, et al. Clinical effect of fasudil hydrochloride for cerebral vasospasm following subarachnoid hemorrhage. Acta Neurochir Suppl 2001;77:209–211

72

Effects of Intrathecal Fibrinolytic Therapy on Clot Lysis and Development of Cerebral Vasospasm in Patients with Aneurysmal Subarachnoid Hemorrhage Following Endovascular Therapy

YOSHIKAZU ARAI, M.D., YUJI HANDA, M.D., PH.D., HISAMASA ISHII, M.D., YOSIFUMI UEDA, M.D., MASAKI ISHIDA, M.D., TAKAHIRO SAKUMA, M.D., TOSHIHIKO KUBOTA, M.D., PH.D.

Abstract

The authors evaluated the efficacy of intrathecal injection of tissue-type plasminogen activator (tPA) for preventing cerebral vasospasm in patients with a diffuse aneurysmal subarachnoid hemorrhage (SAH). The ruptured aneurysm was embolized with Guglielmi detachable coils within 24 hours of SAH. Coil occlusion was followed by immediate intrathecal administration of tPA in eight patients (Hunt and Hess grade 2–4) with diffuse SAH (Fisher grades 2–3) on cranial computed tomography (CT). Each patient received a single injection of tPA (800,000 units) through a spinal intrathecal catheter after which the catheter was clamped for 3 hours. Cerebrospinal fluid was drained through the catheter for up to 10 days. Angiography was performed to evaluate the development of vasospasm 7 to 10 days after SAH. The CT showed complete clearance of the subarachnoid clots in the basal cisterns 24 hours after coil embolization. Residual clot was shown in the insular cisterns or the interhemispheric fissure. No patient developed delayed neurological deficit due to vasospasm and angiography showed only one patient with moderate vasospasm. One patient underwent ventriculoperitoneal shunting for symptomatic hydrocephalus. Most patients, except patients with severe neurological deficits from the onset of SAH, showed excellent to good outcome. Urgent coil embolization of a ruptured aneurysm followed by immediate intrathecal administration of tPA may be a promising therapeutic method in patients with ruptured cerebral aneurysms.

The incidence and severity of cerebral vasospasm after aneurysmal subarachnoid hemorrhage (SAH) are correlated clearly with the amount of clot in the basal cisterns.[1,2] Although experimental and clinical studies have shown that early removal of subarachnoid clot from the basal cisterns decreases the degree of vasospasm and the incidence of severity of delayed ischemic neurological deficits,[3,4] complete mechanical removal of blood clot around the cerebral arteries is difficult in most clinical situations. On the other hand, clot clearance using topical application of tissue-type plasminogen activator (tPA) had shown promising

effects as a method for preventing vasospasm.[5–7] Furthermore, endovascular repair of ruptured aneurysms has been reported to be associated with less risk of delayed ischemic neurological deficit than surgical clipping.[8] These observations led us to evaluate the efficacy of intrathecal injection of tPA following coil embolization of the acutely ruptured aneurysm for prevention of cerebral vasospasm in patients with diffuse SAH.

Clinical Materials and Methods

The first treatment considered for patients with aneurysmal SAH was endovascular coil embolization with Guglielmi detachable coils. Surgical clipping was reserved for patients with aneurysms that were thought to be of suboptimal shape or configuration for coiling. All patients underwent embolization within 24 hours of SAH. After coil embolization, patients who showed dense blood clot in the basal cisterns on cranial computed tomography (CT) underwent placement of a lumbar drain. Continuous lumbar cerebrospinal fluid (CSF) drainage was continued for 3 to 12 days. A single injection of tPA (800,000 units dissolved in 2 mL of saline) was injected into the catheter after which the catheter was closed for 3 hours. Cranial CT scans were obtained every 1 or 2 days after administration of tPA until there was adequate clearance of blood clot from the basal cisterns. Prophylactic hypervolemia or induced hypertension or both were instituted if deemed necessary by the attending neurosurgeon.

This report describes a sequential series of patients treated as just described. There were three males and five females, with a mean age of 64 years. The clinical grade at the time of admission was assessed by the Hunt and Hess grading scale.[9] Four cases were grade 2, two were grade 3, and two were grade 4. The volume of blood clot accumulated in the basal cisterns was graded according to Fisher's scale.[2] There were four patients with Fisher grade 2 SAH and four with grade 3. Seven patients had ruptured anterior circulation aneurysms and one had a dissecting aneurysm of a vertebral artery. All patients underwent magnetic resonance angiography and/or cerebral angiography 2 to 10 days after SAH to evaluate the adequacy of coil embolization and the development of vasospasm. Clinical outcome in each patient was assessed by the Glasgow outcome scale.[10]

Results

Clinical and radiological data are summarized in Table 72–1. Seven saccular aneurysms were completely embolized and one proximal occlusion was performed for a dissecting aneurysm of the vertebral artery. Aneurysms were treated 6 to 20 hours after SAH. Follow-up CT scans showed almost complete clearance of the blood clot in the basal cisterns and

TABLE 72–1 Summary of Clinical and Radiological Characteristics of Eight Patients

Case	Age	Sex	Aneurysm Location	Hunt and Hess Grade	Fisher Grade	Time from SAHto Embolization (hours)	Result (%) of Embolization	Duration of Spinal Drainage	Vasospasm	Hydrocephalus	Glasgow Outcome Score
1	51	Male	Left posterior communicating artery	3	3	14	100	4	No	No	Good recovery
2	88	Female	Right middle cerebral artery	4	3	11	100	4	No	No	Severe disability
3	63	Female	Right anterior cerebral artery	4	3	10	100	12	Yes	Yes	Severe disability
4	53	Male	Anterior communicating artery	2	2	8	100	8	No	No	Good recovery
5	61	Male	Left anterior cerebral artery	2	2	8	100	4	No	No	Good recovery
6	58	Female	Anterior communicating artery	3	2	6	100	5	No	No	Good recovery
7	78	Female	Left posterior communicating artery	2	2	15	100	5	No	No	Good recovery
8	56	Female	Right vertebral artery	2	3	20	Not applicable	3	No	No	Good recovery

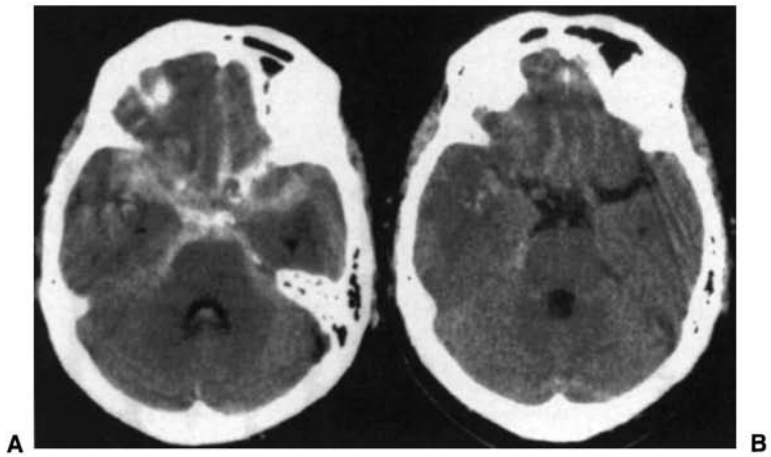
sylvian fissures within 48 hours of tPA administration. All patients who were Hunt and Hess grades 2 and 3 made good recoveries whereas two patients who presented with severe focal neurological deficits on admission and were Hunt and Hess grade 4 remained severely disabled. One patient with a ruptured distal anterior cerebral artery aneurysm showed vasospasm on angiography 7 days after SAH. Seven of eight cases did not develop normal pressure hydrocephalus (see Table 72–1).

Illustrative Case

An 88-year-old woman (case 2, see Table 72–1) presented with decreased level of consciousness and left hemiparesis (Fig. 72–1). The CT revealed thick subarachnoid clot (Fisher grade 3). An aneurysm of the middle cerebral artery bifurcation was successfully embolized with Guglielmi detachable coils 11 hours after SAH. The patient showed gradual improvement in consciousness after lumbar CSF drainage. The CT scan 32 hours after tPA administration showed almost complete clearance of the blood clot in the basal cisterns. The left hemiparesis persisted. Cerebral angiography 7 days after SAH showed no vasospasm of the right side cerebral arteries.

Discussion

It has been recognized that early clot lysis as well as extensive mechanical removal of the subarachnoid clot can reduce the occurrence of chronic vasospasm and delayed ischemic neurological deficits.[1,2] Various technical methods for clot lysis in the acute stage of SAH have been reported. Mizoi et al.[6] reported the effectiveness of multiple intrathecal injections of tPA on development of vasospasm, whereas other studies have described the usefulness of a single injection of tPA administered at the time of craniotomy for aneurysm clipping.[5–7] However, application of tPA at the time of open surgery carries some risk of precipitating serious intra- or postoperative intracranial hemorrhage.



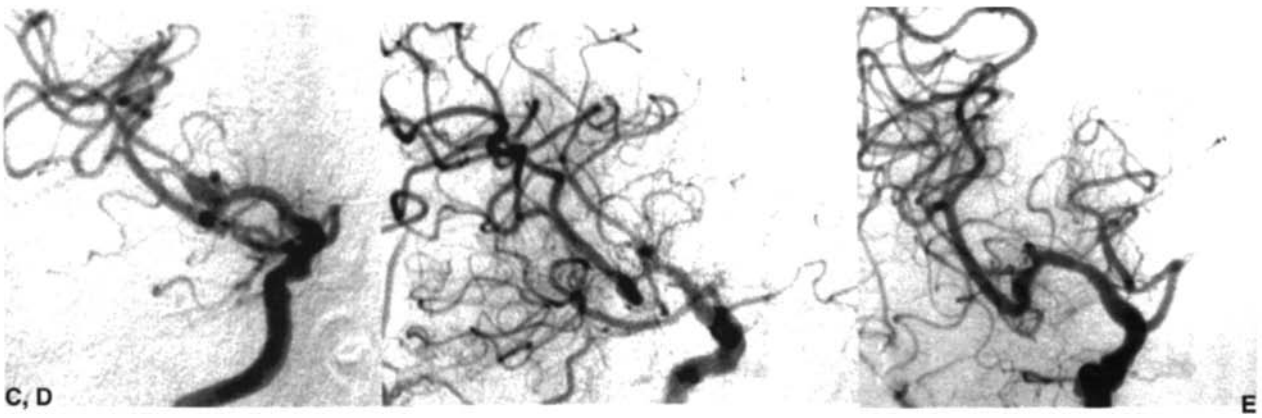


FIGURE 72–1 Neuroimaging in case 2. (A) The computed tomographic scan on admission shows a Fisher grade 3 subarachnoid hemorrhage (SAH). (B) SAH has cleared almost completely by 32 hours after injection of tPA. (C) Angiograms before and (D) after coil embolization of the ruptured right middle cerebral artery aneurysm show adequate coil embolization. (E) Angiogram 7 days after SAH revealed no vasospasm of the right side main cerebral arteries.

Endovascular repair of ruptured aneurysms has been reported to be associated with a lower incidence of delayed ischemic neurological deficits than surgical clipping in patients with SAH. Furthermore, intrathecal tPA application may be at least as safe if not safer in patients who undergo endovascular treatment of the aneurysm instead of clipping. Kinugasa and coworkers reported the successful treatment of ruptured aneurysms by endovascular cellulose acetate polymer thrombosis and intrathecal tPA application.[8] As shown in the present cases, urgent coil embolization followed by lumbar CSF drainage seems to be an important and safe therapeutic strategy for treatment of aneurysmal SAH. Although we used a single injection of tPA at a single dose of 800,000 units in the present series, no tPA-related complications were noted and clot clearance was good. There nevertheless could be a small potential risk that the tPA could destabilize the area of the rupture site. Larger series might examine the issues of tPA dose and method and timing of administration with regard to the potential risk of precipitating aneurysm rebleeding.

Conclusion

Urgent coil embolization of an aneurysm followed by immediate intrathecal administration of tPA may be a promising therapeutic method to prevent development of vasospasm in patients with ruptured cerebral aneurysms.

REFERENCES

1. Fraser J, Johnson S, Ray M, Robertson JT. Prediction of cerebral vasospasm with subarachnoid hemorrhage due to ruptured intracranial aneurysm by computerized tomography. Neurosurgery 1980;6:686–687
2. Kistler JP, Crowell RM, Davis KR, et al. The relation of cerebral vasospasm to the extent and location of subarachnoid blood visualized by CT scan: a prospective study Neurology 1983;33:424–436
3. Handa Y, Weir BKA, Nosko M, Mosewich R, Tsuji T, Grace M. The effect of timing of clot removal on chronic vasospasm in a primate model. J Neurosurg 1987;67:558–564
4. Mizukami M, Kawase T, Usami T, Tazawa T. Prevention of vasospasm by early operation with removal of subarachnoid blood. Neurosurgery 1982;10:301–307
5. Findlay JM, Weir BKA, Kassell NF, Disney LB, Grace MGA. Intracisternal recombinant tissue plasminogen activator after aneurysmal subarachnoid hemorrhage. J Neurosurg 1991;75: 181–188
6. Mizoi K, Yoshimoto T, Takahashi A, Fujiwara S, Koshu K, Sugawara T. Prospective study on the prevention of cerebral vasospasm by intrathecal fibrinolytic therapy with tissue-type plasminogen activator. J Neurosurg 1993;78:430–437
7. Öhman J, Servo A, Heiskanen O. Effect of intrathecal fibrinolytic therapy on clot lysis and vasospasm in patients with aneurysmal subarachnoid hemorrhage. J Neurosurg 1991;75:197–201
8. Kinugasa K, Kamata I, Hirotsune N, et al. Early treatment of subarachnoid hemorrhage after preventing rerupture of an aneurysm. J Neurosurg 1995;83:34–41
9. Hunt WE, Hess RM. Surgical risk as related to time of intervention in the repair of intracranial aneurysms. J Neurosurg 1968; 28:14–20
10. Jennett B, Bond M. Assessment of outcome after severe brain damage: a practical scale. Lancet 1975;1:480–484

73

Intracisternal Thrombolysis with Tissue Plasminogen Activator for Severe Aneurysmal Subarachnoid Hemorrhage

J. MAX FINDLAY, M.D., PH.D.

Abstract

This chapter reviews the author's experience administering recombinant tissue plasminogen activator (rt-PA) intraoperatively to patients suffering large-volume aneurysmal subarachnoid hemorrhages (SAH). Beginning in 1989, all patients with ruptured aneurysms causing thick SAHs as determined by computed tomographic (CT) scanning were considered for intraoperative rt-PA treatment. Except for the first several patients, the standard dose of rt-PA was 10 mg, injected directly into the opened subarachnoid cisterns following aneurysm repair. In addition to clinical observation, postoperative CT scans were obtained within 24 hours to assess clot clearance or new hemorrhage. Cerebral angiography was performed between 6 and 10 days post-SAH to assess vasospasm. Out of a consecutive series of 345 ruptured aneurysms that underwent microsurgical clipping between 1989 and 2002, 54 (16%) were given intracisternal rt-PA. Five patients with thick SAHs were not treated because of an accompanying large intracerebral hemorrhage (three patients), uncertainty of the completeness of aneurysm repair (one patient), and uncertainty that the ruptured aneurysm was repaired in a patient with multiple aneurysms (one patient). There were two postoperative hemorrhages that required reoperation (one extradural and the other from an incompletely clipped aneurysm). Effective "overnight" central cisternal clot clearance was a consistent observation. Severe vasospasm (> 50% narrowing) of a single major artery occurred in three patients, and involvement of more than one major artery in two (9% in total). Mild-to-moderate angiographic vasospasm was detected in another 18 patients (33%). Delayed neurological deterioration primarily due to vasospasm alone occurred in three patients (6%), one of whom underwent angioplasty. In this series of patients with large volume aneurysmal SAH, intracisternal thrombolysis with rt-PA was associated with a relatively low risk of severe angiographic and clinical vasospasm and had an acceptable risk in carefully selected patients.

Experimental studies performed in the late 1980s demonstrated that the fibrinolytic agent recombinant tissue plasminogen activator (rt-PA) is able to lyse subarachnoid hematoma and prevent vasospasm.[1–3] In primates, rt-PA given intrathecally up to 72 hours after subarachnoid hematoma placement significantly reduced vasospasm compared with control preparations.[2] A phase I clinical trial conducted at the University

of Alberta consisting of 15 patients, 13 of whom suffered severe subarachnoid hemorrhage (SAH), was reported in 1991.[4] Tissue plasminogen activator was given as a single intraoperative injection (7.5–15 mg) into the surgically opened subarachnoid cisterns. Mild to moderate (< 50%) vasospasm in at least one major artery was detected in eight patients, no angiographic vasospasm was seen in six patients, and the only patient to develop symptomatic vasospasm died before angiography was performed. One patient experienced a hemorrhagic complication, a large extradural hematoma requiring evacuation within several hours of craniotomy.

Several similar nonrandomized trials also suggested that administration of a fibrinolytic enzyme into the clot-laden subarachnoid space following aneurysm rupture may speed hematoma clearance and reduce cerebral vasospasm with acceptable risks.[5–17] A randomized trial of intraoperative rt-PA for the prevention of vasospasm was reported in 1995,[18] a study of 100 patients from nine centers in Canada and the United States. The treatment protocol consisted of either placebo or 10 mg of rt-PA injected into the subarachnoid cisterns following aneurysm clipping. The incidence of angiographic vasospasm of all degrees did not differ significantly between the treatment and control groups (i.e., the presence or absence of arterial narrowing, regardless of severity), but severe vasospasm (> 50% narrowing) was less frequently observed in the rt-PA group ($p = .02$). Improvement trends in the rt-PA group included a reduced 14-day mortality (6% rt-PA vs 12% placebo) and an improved 90-day outcome (67% rt-PA vs 43% placebo). These differences, however, fell short of statistical significance in this small trial. Bleeding complications were the same between the two groups.

The results of this small randomized trial indicated that rt-PA was useful in reducing the risk of severe angiographic vasospasm, and by extrapolation severe clinical vasospasm, but the data could not prove that this treatment improved overall outcome following aneurysm rupture. Because it did not appear to eliminate the problem of clinical vasospasm following aneurysmal SAH (i.e., it was not the hoped-for "silver bullet"), it is understandable that administration of the clot-dissolving enzyme rt-PA onto the surface of a freshly injured brain at the time of surgery has not become common practice for vasospasm prevention. The author has, however, continued to administer intracisternal rt-PA to selected patients for vasospasm reduction; namely, those suffering large-volume hemorrhages and thick clots in the basal cisterns (Fisher grade 3 hemorrhages).[19] The results of this treatment in a consecutive series of patients are presented here.

Methods

Beginning in 1989, all patients with ruptured intracranial saccular aneurysms associated with thick SAH as determined by computed tomographic (CT) scanning were considered for intraoperative rt-PA treatment. Except for the first several patients, the standard dose of rt-PA has been 10 mg, injected directly into the opened subarachnoid cisterns following aneurysm repair. The rt-PA solution is provided in 1 mg/mL syringes. In addition to clinical observation for symptomatic vasospasm, postoperative CT scans were obtained within 24 hours to assess clot clearance or new hemorrhage, and cerebral angiography between days 6 and 10 post-SAH was done to assess for angiographic vasospasm.

Clinical or symptomatic vasospasm was diagnosed only when cerebral ischemia in the distribution of vasospastic arteries was considered the major reason for the observed neurological deterioration. All other causes of delayed-onset neurological worsening were considered, and the degree of cerebral arterial narrowing was measured with cerebral angiography. Angiographic vasospasm was classified as none to mild (< 25% narrowing compared with baseline, diagnostic angiography), moderate (25–50% narrowing), or severe (> 50%).

Results

Between 1989 and 2002 the author operated on 345 ruptured intracranial saccular aneurysms, 54 (16%) of whom received intracisternal rt-PA. Five (1.4% of the total) with thick subarachnoid clots were not treated with rt-PA, three of whom had more blood clot in the brain parenchyma than in the subarachnoid space. In the remaining two patients treatment was withheld because of uncertainty that the ruptured aneurysm was repaired (one with multiple aneurysms, and the other with a complex aneurysm where it was not clear that the ruptured part of the dome had been fully repaired at the time of surgery).

Significant "overnight" clearance of subarachnoid clot (Fisher grade 3 "thick" hemorrhages reduced to Fisher grade 2 "thin" or no detectable SAH) was seen in 50 (93%) of the treated patients.

Severe (> 50% narrowing compared with baseline vessel caliber) angiographic vasospasm was seen in at least one major cerebral artery in five (9%) patients, moderate vasospasm (25–50% narrowing) was recorded in 11 (20%), and either no or mild vasospasm (0–24% narrowing) was seen in 38 (71%). Symptomatic vasospasm was diagnosed in only three (6%) patients, one of whom underwent angioplasty without clinical benefit.

Significant postoperative intracranial hemorrhages occurred in two patients, one an extradural hematoma that caused early uncal herniation and demanded surgical evacuation, and the other a recurrent SAH from an incompletely clipped aneurysm (proven with postoperative angiography) that also required a second operation. A favorable 1-year outcome was obtained in 42 (78%) of the rt-PA treated patients, the remainder (22%) suffering either death or poor outcome.

Discussion

To put the results of rt-PA treatment for severe SAH into perspective, the author reviewed the results and outcome of 128 patients with Fisher grade 2 (focal and thick clots in the subarachnoid space, but not considered diffuse and "severe" SAH) treated over the same period of time (1989–2002) without intracisternal fibrinolysis, but in an otherwise identical fashion. This comparison is not a cohort study because the Fisher grade 2 patients are not identical patients, and in particular, due to their smaller subarachnoid clot burden have less risk of angiographic and clinical vasospasm.

Moderate to severe angiographic vasospasm was recorded in 34% of these 128 Fisher grade 2 patients, compared with 30% of the 54 Fisher grade 3 patients treated with rt-PA. Symptomatic vasospasm was diagnosed in 10% of the Fisher grade 2 patients, compared with 6% of the rt-PA-treated Fisher grade 3 patients. These differences were not significant, nor were the 1-year outcomes: favorable/poor in 78%/22% of Fisher grade 3 patients and 84%/16% of Fisher grade 2 patients. In summary, the larger group of Fisher grade 2 patients with a smaller vasospasm risk overall was found to have a similar if not greater risk of angiographic or clinical vasospasm than the high-risk Fisher grade 3 group treated with rt-PA.

Although an unpopular preventative measure for vasospasm in North America and Europe due principally to its perceived risk of causing new bleeding, rt-PA lysis of subarachnoid clot has appeared, in the author's experience, to substantially reduce severe angiographic and clinical vasospasm and is quite safe when used after the aneurysm has been fully secured. Given that thick subarachnoid clots cause vasospasm, and thrombolysis reduces clot, this reduction in vasospasm severity following rt-PA treatment is expected and indeed is supported by randomized trials in primates.[1–3] Symptomatic vasospasm is now a relatively infrequent complication of aneurysm rupture, affecting at most 10% of survivors whose aneurysm is repaired.

Until now, the author has used rt-PA for vasospasm prevention only intraoperatively following complete aneurysm clip-repair, so patients with diffuse thick subarachnoid clots due to anterior circulation aneurysm ruptures are generally considered for surgery rather than coil-embolization. The use of fibrinolysis in combination with endovascular treatment of ruptured aneurysms deserves further study

REFERENCES

1. Findlay JM, Weir BKA, Steinke D, et al. Effect of intrathecal thrombolytic therapy on subarachnoid clot and chronic vasospasm in a primate model of SAH. J Neurosurg 1988;69: 723–735
2. Findlay JM, Weir BKA, Gordon P, et al. Safety and efficacy of intrathecal thrombolytic therapy in a primate model of cerebral vasospasm. Neurosurgery 1989;24:491–498
3. Seifert V, Eisert WG, Stolke D, et al. Efficacy of single intracisternal bolus injection of recombinant tissue plasminogen activator to prevent delayed cerebral vasospasm after experimental subarachnoid hemorrhage. Neurosurgery 1989;25:590–598
4. Findlay JM, Weir BKA, Kassell NF, Disney LB, Grace MGA. Intracisternal recombinant tissue plasminogen activator after aneurysmal subarachnoid hemorrhage. J Neurosurg 1991;75: 181–188
5. Kodama N, Sasaki T, Kawakami M, et al. Prevention of vasospasm: Cisternal irrigation therapy with urokinase and ascorbic acid. In: Sano K, Takakura K, Kassell NF, Sasaki T, eds. Cerebral Vasospasm. Proceedings of the IVth International Conference on Cerebral Vasospasm. Tokyo: University of Tokyo Press; 1990:292–296
6. Lamond RG, Alksne JF. Cisternal rt-PA administration in aneurysmal subarachnoid hemorrhage. In: Sano K, Takakura K, Kassell NF, Sasaki T, eds. Cerebral Vasospasm. Proceedings of the IVth International Conference on Cerebral Vasospasm. Tokyo: University of Tokyo Press; 1990:302–303
7. Mizoi K, Yoshimoto T, Fujiwara S, Sugawara T, Takahashi A, Koshu K. Prevention of vasospasm by clot removal and intrathecal bolus injection of tissue-type plasminogen activator: preliminary report. Neurosurgery 1991;28:807–813
8. Mizoi K, Yoshimoto T, Takahashi A, Fujiwara S, Koshu K, Sugawara T. Prospective study on the prevention of cerebral vasospasm by intrathecal fibrinolytic therapy with tissue-type plasminogen activator. J Neurosurg 1993;78:430–437
9. Ohman J, Servo A, Heiskanen O. Effect of intrathecal fibrinolytic therapy on clot lysis and vasospasm in patients with aneurysmal subarachnoid hemorrhage. J Neurosurg 1991;75: 197–201
10. Sasaki T, Ohta T, Kikuchi H, et al. A phase II clinical trial of recombinant human tissue-type plasminogen activator against cerebral vasospasm after aneurysmal subarachnoid hemorrhage. Neurosurgery 1994;35:597–605
11. Seifert V, Eisert WG, Stolke D, Goetz C. Efficacy of single intracisternal bolus injection of recombinant tissue plasminogen activator to prevent delayed cerebral vasospasm after experimental subarachnoid hemorrhage. Neurosurgery 1989;25:590–598
12. Seifert V, Stolke D, Zimmermann M, Feldges A. Prevention of delayed ischaemic deficits after aneurysmal subarachnoid haemorrhage by intrathecal bolus injection of tissue-plasminogen activator (rtPA). Acta Neurochir (Wien) 1994;128:137–143
13. Steinberg GK, Vanefsky MA, Marks MP, Adler JR, Koenig GH. Failure of intracisternal tissue plasminogen activator to prevent vasospasm in certain patients with aneurysmal subarachnoid hemorrhage. Neurosurgery 1994;34:809–814
14. Stolke D, Seifert V. Single intracisternal bolus of recombinant tissue plasminogen activator in patients with aneurysmal subarachnoid hemorrhage: preliminary assessment of efficacy and safety in an open clinical study. Neurosurgery 1992;30:877–888
15. Tanabe T, Arimitsu M, Morimoto M, Kurisaka M, Mori K. The effect of intracranial thrombolytic therapy with tissue-type plasminogen activator on subarachnoid clot and chronic cerebral vasospasm. In: Sano K, Takakura K, Kassell NF, Sasaki T, eds.

Cerebral Vasospasm. Proceedings of the IVth International Conference on Cerebral Vasospasm. Tokyo: University of Tokyo Press; 1990:321–323

16. Usui M, Saito N, Hoya K. Todo T. Vasospasm prevention with postoperative intrathecal thrombolytic therapy: a retrospective comparison of urokinase, tissue plasminogen activator, and cisternal drainage alone. Neurosurgery 1994;34:235–245

17. Zabramski JM, Spetzler RF, Lee KS, et al. Phase I trial of tissue plasminogen activator for the prevention of vasospasm in patients with aneurysmal subarachnoid hemorrhage. J Neurosurg 1991;75:189–196

18. Findlay JM, Kassell NF, Weir BKA, et al. A randomized trial of intraoperative, intracisternal tissue plasminogen activator for the prevention of vasospasm. Neurosurgery 1995;37: 168–178

19. Fisher CM, Kistler JP, Davis KR. The relation of cerebral vasospasm to the extent and location of subarachnoid blood visualized by CT. Neurology 1983;33:424–426

74

Prevention of Vasospasm by Cisternal Irrigation with Urokinase and Ascorbic Acid

TATSUYA SASAKI, M.D., NAMIO KODAMA, M.D., MASATO MATSUMOTO, M.D., KYOUICHI SUZUKI, M.D., JUN SAKUMA, M.D., SONOMI SATO, M.D., MASAHIRO OINUMA, M.D.

Abstract

Cisternal irrigation therapy with urokinase and ascorbic acid has been performed to prevent symptomatic vasospasm in patients with aneurysmal subarachnoid hemorrhage (SAH). Cisternal irrigation with urokinase is used to wash out subarachnoid clot. Ascorbic acid is added to convert oxyhemoglobin, one of the strongest spasmogenic substances, into a nonspasmogenic compound. The efficacy of this therapy was evaluated in 310 consecutive patients. The degree of SAH was classified as Fisher grade 3 and the highest computed tomographic (CT) number of the clot exceeded 60, which suggested a significant risk for symptomatic vasospasm. All patients underwent surgery within 72 hours of SAH. After clipping the aneurysm, irrigation tubes were placed in the sylvian fissure (inlet) and the prepontine cistern (outlet). Lactated-Ringer's solution with urokinase (120 IU/mL) and ascorbic acid (4 mg/mL) was infused at a rate of 30 to 150 mL/hour. Of the 310 patients studied, symptomatic vasospasm was observed in 13 patients (4.2%) and 7 of these patients (2.3%) demonstrated permanent sequelae. The average total drained blood volume was ~112 mL. Analysis of the absorption spectrum of the drainage fluid revealed disappearance of the oxyhemoglobin-specific 576 nm peak with time. Complications occurred in 11 patients during irrigation therapy. These included two patients who experienced seizures, three patients who developed meningitis, and six patients with intracranial hemorrhage. All of these patients recovered without neurological deficits. These results suggest that cisternal irrigation therapy with urokinase and ascorbic acid is effective at preventing symptomatic vasospasm after aneurysmal SAH.

Cerebral vasospasm is one of the most serious complications of aneurysmal subarachnoid hemorrhage (SAH). Although there have been many studies of cerebral vasospasm after SAH, the optimal treatment has not yet been established. We have developed a cisternal irrigation therapy with urokinase and ascorbic acid for the prevention of cerebral vasospasm following acute stage surgery.[1,2] The first goal of this technique is

to dissolve and eliminate the residual subarachnoid clot with urokinase[3] and the second is to change highly spasmogenic oxyhemoglobin[4] into nonspasmogenic verdoheme-like products.[5,6] This study investigated the efficacy of this therapy for preventing symptomatic vasospasm. The surgical procedures and postoperative management in patients undergoing cisternal irrigation therapy are detailed in additional chapters in this volume.

Materials and Methods

During the 19-year period from April 1984 to March 2003, 310 patients with aneurysmal SAH underwent cisternal irrigation with urokinase and ascorbic acid. The degree of SAH was determined on preoperative computed tomographic (CT) scans. Patients were included in this study if they had a Fisher grade 3 SAH on CT[7] and a CT (Hounsfield) number > 60[8] in the highest-density area of the SAH. These CT findings suggest that there is a significant risk for symptomatic vasospasm. All patients underwent acute surgery within 72 hours of SAH. Patient age, sex, ruptured aneurysm location, and preoperative Hunt and Kosnik grade[9] were recorded (Table 74–1).

After craniotomy, the SAH surrounding the internal carotid artery and middle cerebral arteries was removed. Liliequist's membrane was opened and the prepontine clot was aspirated. Following aneurysm clipping, an inlet tube was inserted deeply into the sylvian fissure and a drainage tube was introduced into the prepontine or chiasmatic cistern (Fig. 74–1). Irrigation was bilateral in 33 patients and unilateral in 277.

After surgery, lactated Ringer's solution without urokinase, but with ascorbic acid (4 mg/mL),[6] was infused for 12 hours. Urokinase was not used initially to reduce the risk of postoperative hemorrhage. Urokinase (120 IU/mL)[2,3] was added to the irrigation solution after 12 hours. The original infusion volume was 30 mL/hr/side. Recently, however, the infusion rate was increased to 150 mL/hr so that the irrigation time could be shortened. The irrigation solution was adjusted to the same pH (7.2–7.6) and osmotic pressure (280–300 mOsm/kg) as that of normal cerebrospinal fluid. A microdrop system was used to control the flow rate and a Millipore filter was connected to the infusion tube to prevent infection.

The total volume of infused and drained fluid was measured every hour to avoid excessive infusion. An intracranial pressure control system was usually set at a height of 10 cm H_2O. We counted the red and white blood cells in the drained fluid and examined the level of fibrin degradation products and hemoglobin and the absorption spectrum of the fluid on a daily basis. Irrigation was terminated based on the number of days from SAH onset, the absence of a high-density area on CT near the sylvian fissures, and data from drained fluid (red blood cells and fibrin degradation products < 10,000/mm^3 and 10 μg/mL, respectively). The duration of cisternal irrigation ranged from 2 to 18 days with a mean of 10 days.

Results

Among the 310 patients, all of whom were at high risk of symptomatic vasospasm, only 13 (4%) suffered symptomatic vasospasm. Sequelae occurred in seven of these 13 patients (2%). The outcome assessed at discharge from the hospital was excellent in 177 cases (57%), good in 73 (24%), fair in 35 (11%), and poor in 18 (6%, Table 74–2). Seven patients (2%) died, resulting in morbidity and mortality rates of 17% and 2%, respectively. None of the seven patients who died manifested symptoms of vasospasm.

TABLE 74–1 Summary of 310 Patients Treated with Urokinase and Ascorbic Acid

Characteristic		**Number of Patients** (%)
Mean age (years)		60 (range: 27–87)
Sex	Male	121
	Female	189
Site of aneurysm	Anterior communicating artery	120
	Internal carotid artery	93
	Middle cerebral artery	75
	Vertebrobasilar system	15
	Anterior cerebral artery	7
Preoperative Hunt and Kosnik grade[9]	1	6
	2	138
	3	104
	4	61
	5	1

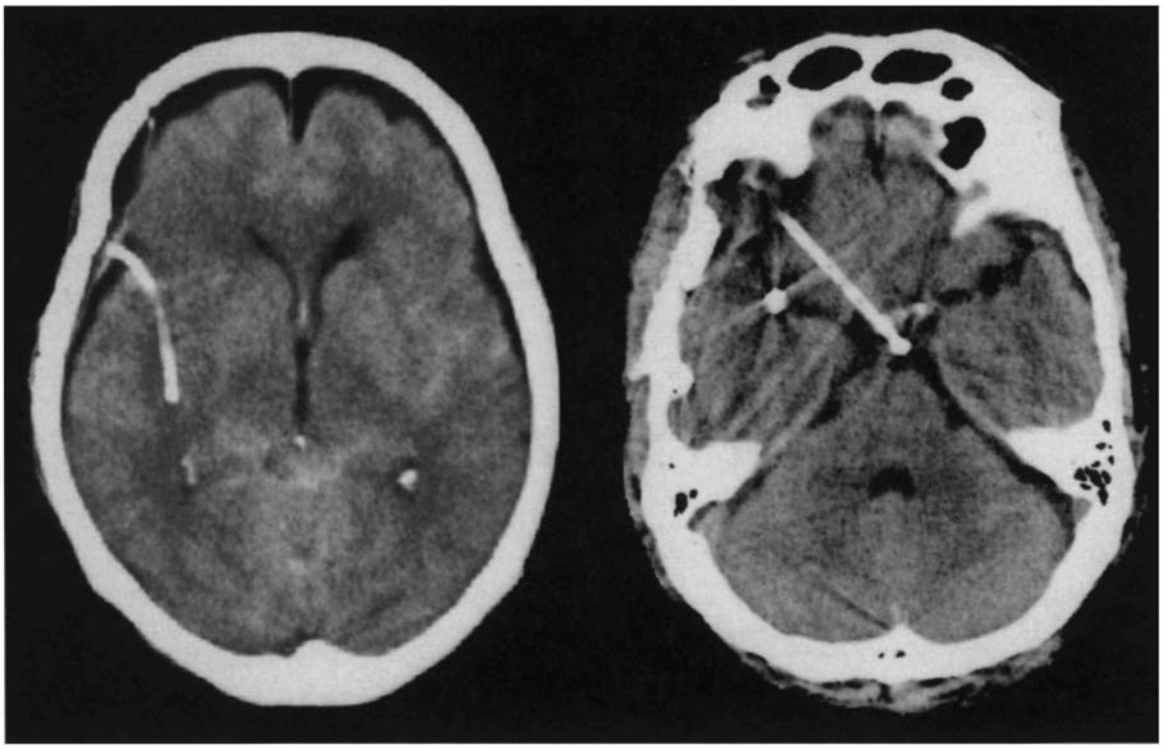


FIGURE 74–1 Computed tomographic scans showing the position of the irrigation tubes. The inlet tube is positioned in the sylvian fissure (left) and the outlet tube was introduced into the prepontine cistern (right).

Of the 310 patients treated by irrigation, 11 (4%) experienced complications. Two patients (0.6%) suffered from seizures, three (1%) developed meningitis, and six (2%) developed intracranial hemorrhage. In both patients with seizures, the drainage tube, introduced into the subdural space, became occluded. Upon pulling its tip out from the subdural to the epidural space, the drainage flow again became smooth. Neither of these patients suffered from symptomatic vasospasm. Three patients with infectious meningitis recovered completely following bilateral ventricular catheterization and irrigation with antibiotic agents. The intracranial bleeding in six patients was intracerebral in four and subdural in two. Two hematomas were evacuated surgically. All six patients with postoperative hemorrhage were discharged from the hospital without any neurological deficits. None of these complications led to morbidity or death.

Sequential changes in the erythrocyte count in the drained fluid revealed an increase immediately after urokinase delivery, followed by a gradual decrease starting on day 2. Changes in fibrin degradation product levels and the white blood cell count were similar to those seen with the red blood cells. The cell-free hemoglobin levels (in the supernatant fluid) decreased shortly after the start of irrigation and manifested a

TABLE 74–2 Preoperative Hunt and Kosnik Grade and Clinical Outcome in 310 Patients Treated with Cisternal Irrigation*

	Outcome					
Hunt and Kosnik Grade	**Excellent**	**Good**	**Fair**	**Poor**	**Dead**	**Total**
1	**4**	**2**	**0**	**0**	**0**	**6**
2	100 **(2)**	25(1)	**9(2)**	**3(1)**	**1**	138 **(6)**
3	47(1)	34(1)	**14(1)**	**6(2)**	**3**	104 **(5)**
4	**26**	**12**	**12**	**9(2)**	**2**	61(2)
5	**0**	**0**	**0**	**0**	**1**	**1**
Total	177 **(3)**	73(2)	35(3)	18(5)	**7**	310 **(13)**

*Numbers in parentheses are patients with symptomatic vasospasm.

smaller secondary increase on postoperative days 6 and 7. The total drained blood volume was 112 ± 15 mL (mean ± standard error of the mean). Although many patients showed proliferation of white blood cells in the drainage fluid, there were no signs of left shift or systemic inflammation. Bacterial cultures were negative except in patients with meningitis. The cell count in the drainage fluid decreased rapidly after discontinuation of the irrigation.

Analysis of the absorption spectrum of the drainage fluid disclosed a gradual decline and disappearance of the peak at 576 nm that is specific for oxyhemoglobin by day 5.[1,6,10] When we compared these results with those obtained in patients treated by cisternal drainage alone, we noted that in the latter patients, there was an increase in the oxyhemoglobin peak after day 3 that reached its highest value on day 9 and remained higher than in irrigated patients until day 13.[1,10]

Discussion

Based on data gleaned from experimental studies, we have performed cisternal irrigation using urokinase and ascorbic acid after acute-stage surgery for aneurysmal SAH since 1984.[1,2,5,6,9] Our attempt was not only to dissolve and eliminate the residual blood clot by nonmechanical means but also to change the spasmogenic substances in the residual clot into nonspasmogenic substances, paying special attention to oxyhemoglobin,[4] a potent vasoconstrictor. The present study included only patients with severe SAH, defined by the classification of Fisher et al[7] and the CT (Hounsfield) number. These patients were thought to be at significant risk for vasospasm. Of the 310 patients included in our study, only 13 (4%) developed symptomatic vasospasm, and 7 (2%) suffered from sequelae of vasospasm. According to a literature review of more than 30,000 cases by Dorsch and King,[11] symptomatic vasospasm or delayed ischemic deficit occurred in 33% of patients with aneurysmal SAH. Of the patients with delayed ischemic deficits, 30% died and 34% suffered permanent neurological deficits. In our series, 3% of irrigated patients manifested morbidity due to cerebral vasospasm and none of these patients died. Our results demonstrate that cisternal irrigation therapy with urokinase and ascorbic acid effectively prevents symptomatic vasospasm in patients with aneurvsmal SAH. The complication rate amone patients treated by cisternal irrigation was 4% (n = 11), although none of these patients developed permanent morbidity or died.

We administered irrigation therapy to all patients at high risk for vasospasm because it is impossible to predict which of these patients will progress to symptomatic vasospasm. Studies are under way in our laboratory to identify a group of patients at highest risk for vasospasm.

Some problems must be solved before cisternal irrigation can be used on a broad scale. The most appropriate treatment duration remains to be determined. At present, the mean treatment period is 10 days. The care of patients during this time is very labor intensive on the part of the medical staff. However, in our institution the riskibenefit ratio of cisternal irrigation is extremely high because only seven patients (2%) developed symptomatic vasospasm that resulted in permanent sequelae.

REFERENCES

1. Kodama N, Sasaki T, Kawakami M, Sato M, Asari J. Cisternal irrigation therapy with urokinase and ascorbic acid for prevention of vasospasm after aneurysmal subarachnoid hemorrhage: outcome in 217 patients. Surg Neurol 2000;53:110–118
2. Sasaki T, Kodama N, Kawakami M, et al. Urokinase cisternal irrigation therapy for prevention of symptomatic vasospasm after aneurysmal subarachnoid hemorrhage: a study of urokinase concentration and the fibrinolytic system. Stroke 2000;31: 1256–1262
3. Yamanobe K. Prevention of vasospasm: experimental studies on lysis of urokinase. Fukushima Med J 1987;37:27–39
4. Osaka K. Prolonged vasospasm produced by the break-down products of erythrocytes. J Neurosurg 1977;47:403–411
5. Kawakami M, Kodama N, Toda N. Suppression of the cerebral vasospastic actions of oxyhemoglobin by ascorbic acid. Neurosurgery 1991;28:33–40
6. Sato M. Prevention of cerebral vasospasm: experimental studies on the degradation of oxyhemoglobin by ascorbic acid. Fukushima J Med Sci 1987;33:55–70
7. Fisher CM, Kistler JR, Davis JM. Relation of cerebral vasospasm to subarachnoid hemorrhage visualized by computerized tomographic scanning. Neurosurgery 1980;6:1–9
8. Suzuki J, Komatsu S, Sato T, Sakurai Y. Correlation between CT findings and subsequent development of cerebral infarction due to vasospasm in subarachnoid hemorrhage. Acta Neurochir (Wien) 1980;55:63–70
9. Hunt WE, Kosnik EJ. Timing and perioperative care in intracranial aneurysm surgery. Clin Neurosurg 1974;21:79–89
10. Konno Y, Sato T, Suzuki K, Matsumoto M, Sasaki T, Kodama N. Sequential changes of oxyhemoglobin in drained fluid of cisternal irrigation therapy: reference of the effect of ascorbic acid. Acta Neurochir Suppl 2001;77:167–169
11. Dorsch NWC, King MT. A review of cerebral vasospasm in aneurysmal subarachnoid haemorrhage. J Clin Neurosci 1994;1: 19–26

75

Prevention of Vasospasm: Surgical Procedures and Postoperative Management in Patients Undergoing Cisternal Irrigation

NAMIO KODAMA, M.D., TATSUYA SASAKI, M.D., MASATO MATSUMOTO, M.D., KYOUICHI SUZUKI, M.D., JUN SAKUMA, M.D., SONOMI SATO, M.D., AND MASAHIRO OINUMA, M.D.

Abstract

Cisternal irrigation therapy with urokinase and ascorbic acid has been performed to prevent symptomatic vasospasm after aneurysmal subarachnoid hemorrhage (SAH). The procedure involves a craniotomy followed by initial evacuation of SAH around the internal carotid artery and sphenoidal segment of the middle cerebral artery. The prepontine clot is aspirated after opening Liliequist's membrane. After clipping the aneurysm, a space for inserting a tube is developed in the sylvian fissure. An inlet tube is placed in the space. A drainage tube is placed in the prepontine cistern. Immediately after surgery, lactated Ringer's solution with ascorbic acid (4 mg/mL) is infused. Urokinase (120 IU/mL) is added 12 hours after surgery This delay is to reduce the risk of postoperative hemorrhage. Infusion volume is 30 to 150 mL/hour. Total volumes of infused and drained fluids are measured every hour to avoid excessive infusion. Red and white blood cells, fibrin degradation products, hemoglobin (in the supernatant fluid), absorption spectrum of oxyhemoglobin, pH, and osmotic pressure are measured every day on the drained fluid. Termination of the therapy is determined by the number of days from SAH, computed tomographic (CT) findings, and data from the drained fluid. Of the 310 patients studied, symptomatic vasospasm was observed in 13 patients (4%). Seven of these patients (2%) demonstrated sequelae. These results suggest that cisternal irrigation therapy with urokinase and ascorbic acid is effective in preventing symptomatic vasospasm after aneurysmal SAH. Although patients are bedridden for an average of 10 days and the management of the drainage system is complex and difficult, the benefit from this therapy appears to far exceed its disadvantages.

We have performed cisternal irrigation with urokinase and ascorbic acid to prevent cerebral vasospasm following acute-stage surgery in patients with subarachnoid hemorrhage (SAH) since 1984.[1,2] Urokinase is used to dissolve the residual clot[3] and ascorbic acid is added to break down oxyhemoglobin, one of the putative causes of vasospasm,[4,5] into nonspasmogenic verdoheme-like products.[6,7] This chapter details our

surgical procedures and postoperative management of patients undergoing cisternal irrigation therapy. The patient population and clinical results are reported separately by Sasaki et al in this volume (chapter 74).

Materials and Methods

Cisternal irrigation therapy with urokinase and ascorbic acid was performed in 310 patients to prevent vasospasm after aneurysmal SAH. The degree of their SAH was determined on preoperative computed tomographic (CT) scans. Patients were included in the study if the admission CT scan showed a Fisher grade 3 SAH[8] and there was a CT (Hounsfield) number > 60[9] in at least some part of the SAH. These CT findings suggest a significant risk for symptomatic vasospasm. All patients underwent surgery within 72 hours of SAH.

For patients with supratentorial aneurysms, craniotomy was performed and brain relaxation was usually achieved by opening Liliequist's membrane and aspirating the clot in the prepontine cistern. Third ventriculostomy or ventricular tapping was also performed in some cases to achieve an adequately slack brain. Removal of the SAH surrounding the internal carotid artery and the sphenoidal and proximal parts of the insular segment of the middle cerebral artery was then performed. After aneurysmal clipping, an inlet tube was introduced into the deep portion of the sylvian fissure. A drainage tube was placed in the prepontine or chiasmatic cistern. For patients with infratentorial aneurysms, the aneurysm was clipped with the patient in the prone or lateral position. Following clipping, the patient was turned supine and a small craniotomy was performed in the frontotemporal region on the right side. The sylvian fissure was opened and an infusion tube was inserted. A drainage tube was placed in the prepontine or chiasmatic cistern.

After surgery, lactated Ringer's solution without urokinase, but with ascorbic acid (4 mg/mL), was infused for 12 hours. Urokinase (120 IU/mL) was added to the irrigation solution after 12 hours. The solution was adjusted to the same pH (7.2–7.6) and osmotic pressure (280–300 mOsm/kg) as normal cerebrospinal fluid. The infusion volume was originally 30 mL/hr but has been increased to 150 mL/hr recently to reduce the duration of irrigation required to clear the SAH. A microdrop system was used to control the flow rate and a Millipore filter was connected to the infusion tube to prevent infection. An intracranial pressure control system was usually set at a height of 10 cm H_2O (Fig. 75–1). Total volumes of infused and drained fluid were measured every hour to avoid excessive infusion. Red and white blood cells, fibrin degradation products, hemoglobin in the supernatant fluid, and the absorption spectrum in the drained fluid were measured daily Irrigation therapy was stopped based on the number of days since SAH, the absence on CT of a high-density area near the sylvian fissure, and data from the drained fluid (erythrocyte count and fibrin degradation products < 10,000/mm^3 and 10 |xg/mL, respectively). The duration of cisternal irrigation therapy ranged from 2 to 18 days with a mean of 10 days. Irrigation was bilateral in 33 and unilateral in 277 patients. We performed unilateral cisternography with ^{99m}technetium-diethylenetriamine pentaacetic acid (^{99m}Tc-DTPA) and noted that there was diffusion of the ^{99m}Tc-DTPA over both hemispheres after unilateral infusion (Fig. 75–2). This prompted us to change from bilateral to only unilateral irrigation even in patients with bilateral SAH.

Results

Of the 310 patients studied, 13 (4%) suffered symptomatic vasospasm; and in 7 of these (2%) there were sequelae. The morbidity and mortality rates were 20% and 2%, respectively. None of the seven patients who died had symptoms of vasospasm. There were complications of irrigation therapy in 11 patients. Two patients (0.6%) had seizures, three (1%) developed meningitis, and six (2%) suffered intracranial hemorrhage. In both patients with seizures, the drainage tube had been placed in the subdural space. Its occlusion resulted in increased intracranial pressure. After the tip of the tube was pulled from the subdural into the epidural space the drainage flow again became smooth and neither patient suffered vasospasm. All three patients who contracted infections recovered completely following bilateral ventricular catheterization and irrigation with antibiotic agents. The patients with intracranial bleeding included four with intracerebral hemorrhage and two with subdural hematomas. In the patients with intracranial hemorrhage, there was noted to be an increase in the erythrocyte count in the drainage fluid along with sudden severe headache. Cranial CT scans showed new intracranial bleeding and irrigation was stopped immediately Hematologic tests did not show systemic hypofibrinogenemia or hemorrhagic risk factors in any of the six patients. Two of the six underwent hematoma evacuation. All patients with intracerebral hemorrhage were discharged from the hospital without any neurological deficits. None of the complications we encountered led to morbidity or death.

The fluid drained from each patient was examined every day for the infused and drained volume, the number of erythrocytes, and the level of fibrin degradation products, hemoglobin, and oxyhemoglobin (Table 75–1). The erythrocyte counts in the drained fluid revealed an increase immediately after the

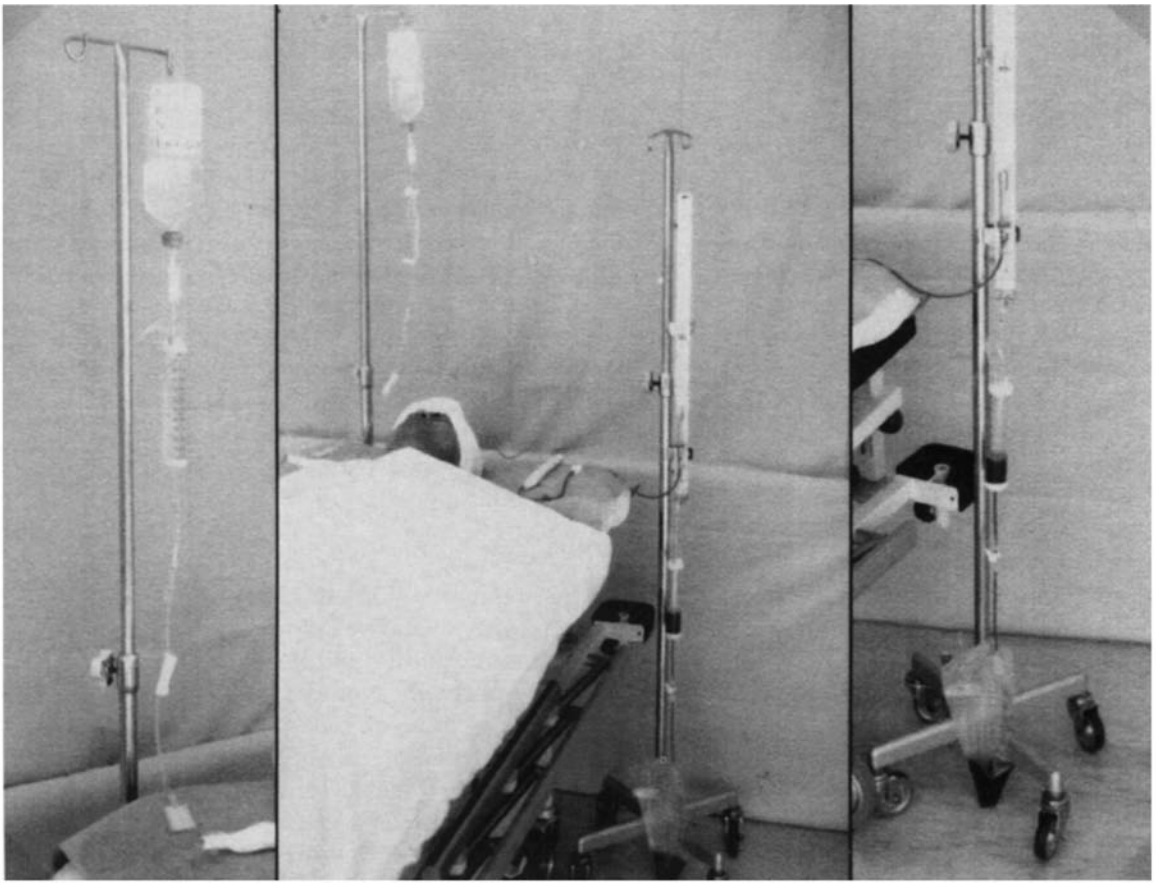


FIGURE 75–1 Patient undergoing cisternal irrigation. A microdrop system (left) is used to control the flow rate. There is a Millipore filter connected to the tube to prevent infection. Intracranial pressure was usually maintained at 10 cm H_2O by manipulating the position of the outflow tube (right).

administration of urokinase and then a gradual decrease starting on the second day. Changes in the level of fibrin degradation products paralleled those of the red blood cell counts. The cell-free (supernatant fluid) hemoglobin levels decreased shortly after the start of irrigation, and there was a small secondary increase on the sixth and seventh postoperative days. The average total drained blood volume was 112 mL. In many patients white blood cells proliferated in the drainage fluid, although there were no signs of left shift or of systemic inflammation. Bacterial cultures were negative except in patients with meningitis. The cell count in the drainage fluid decreased rapidly after irrigation was discontinued.

Analysis of the absorption spectrum of the drainage fluid disclosed disappearance of the peak at 576 nm that is specific for oxyhemoglobin. Analysis of mean values of the absorption spectrum revealed that the oxyhemoglobin-specific peak decreased by day 5 and remained low in the irrigation group. We compared these results with those obtained in patients undergoing cisternal drainage only. In contrast to the irrigation group, the oxyhemoglobin peak in the drainage group increased after day 3, reached its highest value on day 9, and remained higher than in the irrigation group until day 13.

Discussion

We have selected for irrigation therapy only patients suggested to be at significant risk for vasospasm. The criteria were severe SAH defined as CT grade 3 according to the classification of Fisher et al[8] and a CT (Hounsfield) number > 60.[9] Of the 310 patients thus selected, 13 (4%) developed symptomatic vasospasm and only 7 (2%) suffered permanent sequelae of vasospasm. These results demonstrate that cisternal irrigation with urokinase and ascorbic acid is effective in preventing symptomatic vasospasm in patients with aneurysmal SAH. Based on our experience in 11 patients undergoing cisternal irrigation who developed complications, we suggest that the drainage tube should be placed in the cisterns rather than the subdural space because the former offers more space. If the drainage tube becomes occluded, irrigation ceases. We observed the proliferation of white blood

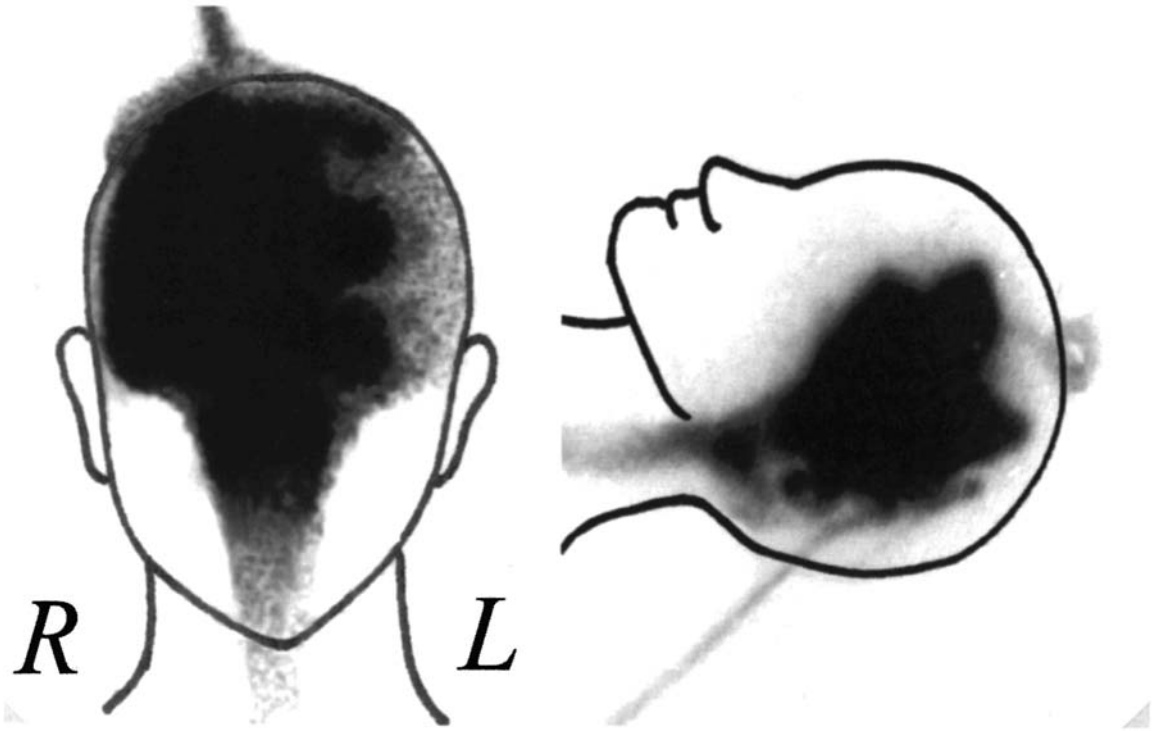


FIGURE 75–2 (left) Cisternogram (anteroposterior view) showing bilateral spread of ^{99m}technetium-diethylenetriamine pentaacetic acid (^{99m}Tc-DTPA) after only unilateral irrigation was performed, (right) Cisternogram with ^{99m}Tc-DTPA (lateral view) showing spread of the tracer to the posterior fossa and spinal cord.

cells in the cerebrospinal fluid during irrigation. This may be regarded as chemical meningitis induced by urokinase or ascorbic acid or both, based on the observations that there were no signs of systemic inflammation, that bacterial cultures were negative, and that cell counts in the drained fluid decreased rapidly upon discontinuation of irrigation. The risk of infection should not be overlooked, however. The system used was open and in fact several patients did develop infections.

Six patients suffered intracranial hemorrhages possibly attributable to iatrogenic injury, minor local trauma to the brain and small vessels near the irrigation tubes,

TABLE 75–1 Results of Examination of Drainage Fluid.

	In–Out (mL)		Erythrocytes (/mm^3)	Hemoglobin (mg/dL)	White Blood Cells (/mm^3)	Fibrin Degradation Products (µg/mL)	Oxyhemoglobin Absorption	Blood Volume (mL)	
Date	**In**	**Out**						**Per Day**	**Total**
January 26	86	136	87000	34.2	3170	80	0.087	3.1	3.1
January 27	396	776	73500	8.2	2950	40	0.028	13.6	16.7
January 28	506	727	99500	4.5	1350	160	0.016	17.0	33.6
January 29	540	623	144000	1.4	1240	160	0.023	20.7	54.3
January 30	581	818	156500	1.1	790	80	0.026	29.6	83.9
January 31	428	769	120500	0.7	590	80	0.018	21.4	105.3
February 1	486	748	120000	0.9	609	80	0.019	20.7	126.0
February 2	546	735	100500	1.3	586	80	0.038	17.1	143.1
February 3	467	705	48500	0.2	322	40	0.050	7.9	151.0
February 4	504	754	13000	1.5	378	20	0.032	2.3	153.3
February 5	434	741	6050	1.4	441	20	0.019	1.1	154.5
February 6	538	833	1600	0.9	115	10	0.011	0.4	154.8

and/or the fibrinolytic activity of the urokinase. Two of these patients required hematoma evacuation. Clearly, there is some risk of hemorrhage with irrigation therapy. None of the complications, however, resulted in morbidity or death and we suggest that cisternal irrigation therapy with urokinase and ascorbic acid is effective and safe in preventing symptomatic vasospasm after aneurysmal SAH. Although patients undergoing irrigation had to remain hospitalized for as long as 10 days and their care was very labor intensive during the irrigation therapy, we suggest that the benefits derived from cisternal irrigation therapy far exceeded any of the difficulties that may be encountered.

REFERENCES

1. Kodama N, Sasaki T, Kawakami M, Sato M, Asari J. Cisternal irrigation therapy with urokinase and ascorbic acid for prevention of vasospasm after aneurysmal subarachnoid hemorrhage: outcome in 217 patients. Surg Neurol 2000;53:110–118
2. Sasaki T, Kodama N, Kawakami M, et al. Urokinase cisternal irrigation therapy for prevention of symptomatic vasospasm after aneurysmal subarachnoid hemorrhage: a study of urokinase concentration and the fibrinolytic system. Stroke 2000;31:1256–1262
3. Yamanobe K. Prevention of vasospasm: experimental studies on lysis of urokinase. Fukushima Med J 1987;37:27–39
4. Osaka K. Prolonged vasospasm produced by the break-down products of erythrocytes. J Neurosurg 1977;47:403–411
5. Konno Y, Sato T, Suzuki K, Matsumoto M, Sasaki T, Kodama N. Sequential changes of oxyhemoglobin in drained fluid of cisternal irrigation therapy: reference of the effect of ascorbic acid. Acta Neurochir Suppl 2001;77:167–169
6. Kawakami M, Kodama N, Toda N. Suppression of the cerebral vasospastic actions of oxyhemoglobin by ascorbic acid. Neurosurgery 1991;28:33–40
7. Sato M. Prevention of cerebral vasospasm: experimental studies on the degradation of oxyhemoglobin by ascorbic acid. Fukushima J Med Sci 1987;33:55–70
8. Fisher CM, Kistler JR, Davis JM. Relation of cerebral vasospasm to subarachnoid hemorrhage visualized by computerized tomographic scanning. Neurosurgery 1980;6:1–9
9. Suzuki J, Komatsu S, Sato T, Sakurai Y. Correlation between CT findings and subsequent development of cerebral infarction due to vasospasm in subarachnoid hemorrhage. Acta Neurochir (Wien) 1980;55:63–70

76

Prevention of Symptomatic Vasospasm by Intermittent Cisternal Irrigation with Urokinase and Ascorbic Acid

HIROYUKI MURAMATSU, M.D., TAKU SATO, M.D., MASAHIRO OINUMA, M.D., KYOUICHI SUZUKI, M.D., MASATO MATSUMOTO, M.D., TATSUYA SASAKI, M.D., NAMIO KODAMA, M.D.

Abstract

Intermittent cisternal injection therapy with urokinase and ascorbic acid was introduced to prevent symptomatic vasospasm after aneurysmal subarachnoid hemorrhage (SAH). Urokinase was administered to dissolve and wash out the subarachnoid clot and ascorbic acid was added to break down oxyhemoglobin, one of the putative spasmogenic substances responsible for vasospasm, into verdoheme-like products that are nonspasmogenic. The efficacy and safety of this therapy was evaluated in 20 patients with severe SAH. Lactated Ringer's solution (10 mL) with urokinase (300 IU/mL) and ascorbic acid (10 mg/mL) was injected via a cisternal drainage tube twice a day There were no cases of symptomatic cerebral vasospasm and no complications were attributed to the treatment. The average total drained blood volume calculated from the drainage fluid was 41 mL. Analysis of the spectrophotometric absorption spectrum of the drainage fluid revealed a decrease in the peak at 576 nm that is specific for oxyhemoglobin. The results suggest that intermittent cisternal injections of urokinase and ascorbic acid may be effective in preventing symptomatic vasospasm. Further studies are needed to determine indications for intermittent injection therapy, the concentrations of urokinase and ascorbic acid to use, the volume of fluid to inject, and the frequency of injection.

Cerebral vasospasm is one of the most serious complications of aneurysmal subarachnoid hemorrhage (SAH). In the absence of definitive treatments, its prevention remains an important goal. We previously reported that cisternal irrigation with urokinase and ascorbic acid effectively prevents symptomatic vasospasm after SAH in patients who are operated on acutely.[1,2] Urokinase is used to dissolve the residual clot and ascorbic acid is added to break down oxyhemoglobin,[3] a key spasmogen believed to contribute to development of vasospasm.[4,5] In this study we evaluated the effectiveness of intermittent cisternal irrigation with urokinase and ascorbic acid for preventing symptomatic vasospasm.

Materials and Methods

During the 4-year period from January 1999 to December 2002, 20 patients (8 men, 12 women) ranging in age from 45 to 84 years (mean 61 years) received intermittent cisternal irrigation therapy with urokinase and ascorbic acid. The preoperative Hunt and Kosnik grade was 2 in 12 patients and 3 in eight patients.[6]

We arrived at the decision to perform intermittent irrigation based on the following considerations. Patients were classified as Fisher grade 3[7] but did not satisfy our additional requirement for cisternal irrigation in that the computed tomographic (CT, Hounsfield) number did not exceed 60.[8] In addition, cisternal drainage, which was used in these cases, produced unexpectedly poor clot washout (n = 14). In other cases/the attending physician chose to perform cisternal drainage although they did satisfy our cisternal irrigation criteria (n = 3), postoperative CT disclosed an increase in the SAH volume due to intraoperative rupture (n = 2), or the irrigation tube in the sylvian fissure was occluded (n = 1).

The cisternal drainage tube was placed into the prepontine cistern in 17 patients with supratentorial aneurysms and into the cisterna magna in three with infratentorial aneurysms. Lactated Ringer's solution (10 mL) containing urokinase (300 IU/mL) and ascorbic acid (10 mg/mL) and adjusted to the same pH (7.2–7.6) and osmotic pressure (280–300 mOsm/kg) as normal cerebrospinal fluid was injected twice a day via the cisternal drainage tube. The drainage tube was clamped for 1 hour after each injection. Intracranial pressure was usually maintained at 10 cm H_2O. Red

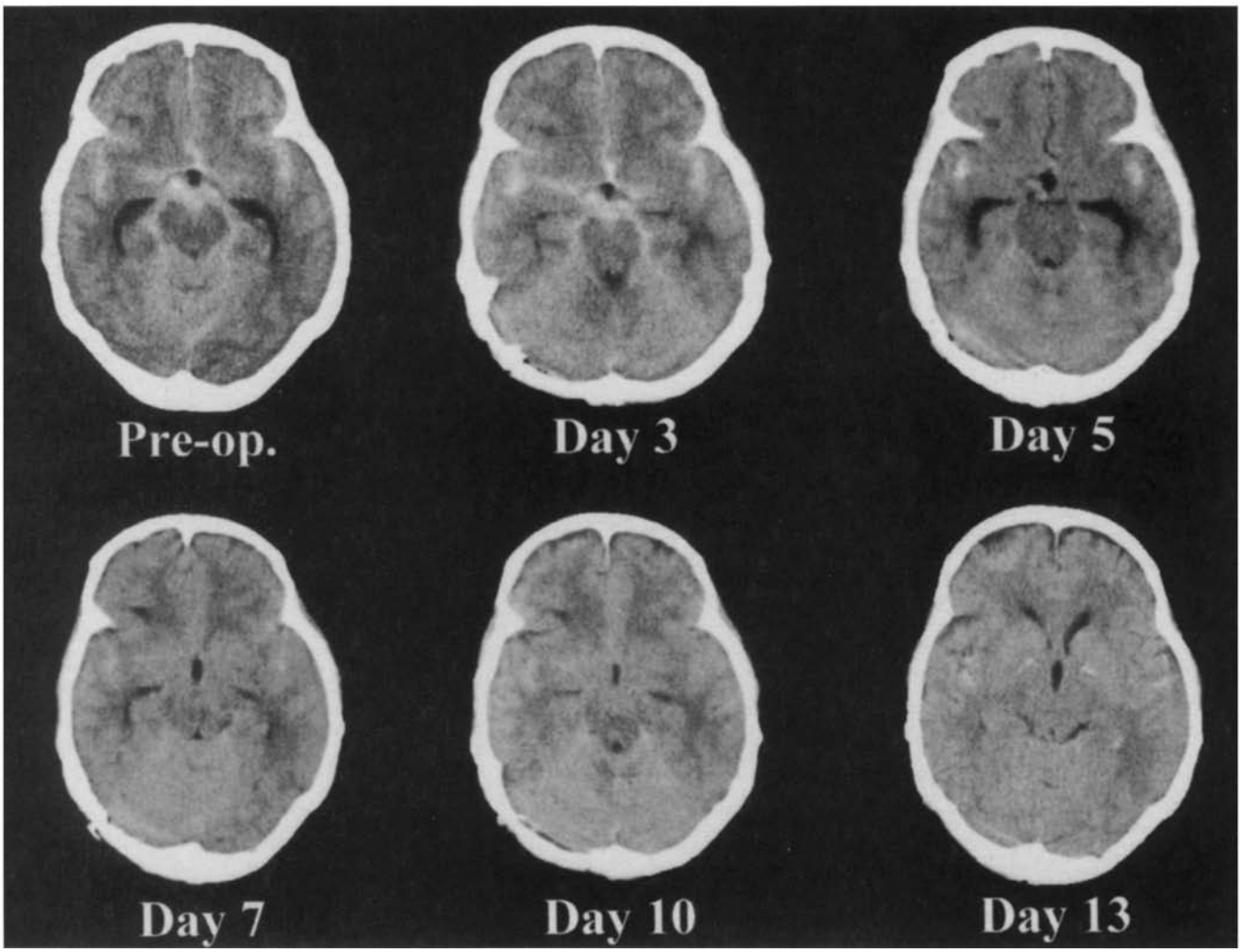


FIGURE 76–1 Computed tomographic (CT) scans from a representative patient treated with intermittent cisternal injections of urokinase and ascorbic acid. The patient was a 47-year-old woman who underwent trapping of a left vertebral dissecting aneurysm. The drainage tube was placed in the cisterna magna. A CT scan obtained on day 3 after subarachnoid hemorrhage (SAH) revealed persistence of her subarachnoid hematoma in both sylvian fissures. On this basis, intermittent irrigation was performed from days 3 to 7. She did not develop symptomatic vasospasm.

blood cells, fibrin degradation products, and the hemoglobin concentration (in the supernatant portion) in the drained fluid were measured daily and the total drained blood volume was calculated. The absorption spectrum of the drained fluid was analyzed spectrophotometrically and sequential changes were recorded. Data on the drained fluid from patients undergoing intermittent irrigation, continuous cisternal irrigation, and simple cisternal drainage were compared.

Results

Irrigation was terminated based on the number of days from SAH onset, the absence of a high-density area on CT scans, and the finding that the erythrocyte count and concentration of fibrin degradation products in the drained fluid were below 10,000/mm^3 and 10 μg/mL, respectively. The duration of intermittent irrigation ranged from 5 to 10 days (mean 7.5 days). Sequential changes seen on CT scans of a representative case are shown in Figure 76–1.

None of the 20 patients who underwent intermittent irrigation experienced symptomatic vasospasm or complications such as meningitis or hemorrhage. At the time of discharge, the activity of daily life score was 1 for 17 patients, 2 for two patients, and 3 for one patient. The mean volume of fluid drained in the intermittent irrigation group was higher (41 mL) than in patients subjected to drainage only (23 mL) but the difference was not significant (Fig. 76–2). The volume was, however, significantly lower than in patients with cisternal irrigation (112 mL, $p < .05$).

As shown in Figure 76–3, the mean values of the absorption spectrum peak at 576 nm that corresponds to a specific absorption peak for oxyhemoglobin were higher in patients subjected to intermittent irrigation than in those undergoing continuous irrigation. The values were lower than in patients treated by drainage alone. These differences were not statistically significant.

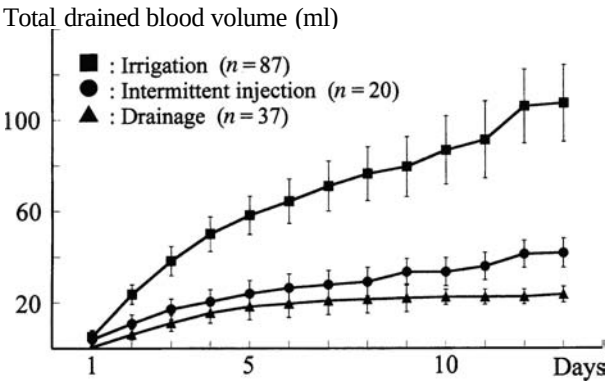


FIGURE 76–2 Total drained blood volume by day after subarachnoid hemorrhage. The volumes were calculated from the red blood cell counts and hemoglobin concentration in the supernatant component of the drained fluid (values are means ± standard error of the mean). The mean total drained blood volume was 112 mL in the continuous irrigation group, 23 mL in the drainage-only group, and 41 mL in the intermittent irrigation group.

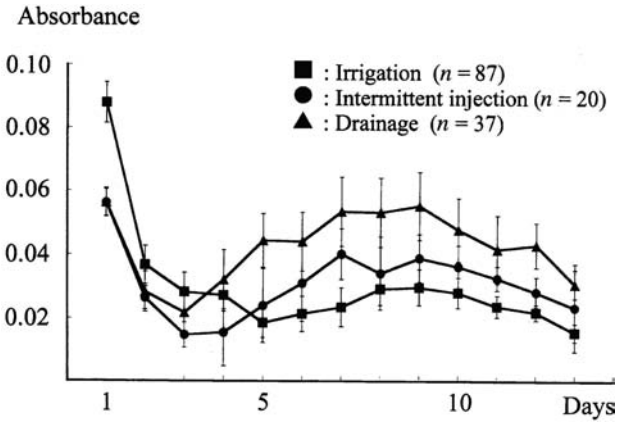


FIGURE 76–3 Mean values of the absorbance of the drainage fluid at 576 ran, which is a wavelength that has a peak specific for oxyhemoglobin. Values are means ± standard errors of the means and are shown for the continuous irrigation, drainage only, and intermittent irrigation groups. The mean value of the intermittent irrigation group was lower than in the drainage-only and higher than in the continuous irrigation group.

Discussion

We perform cisternal irrigation with urokinase and ascorbic acid to prevent symptomatic vasospasm in patients with SAH.[1,2] To be eligible for irrigation, patients must undergo surgery within 72 hours of SAH, the SAH on preoperative CT scan must be grade 3 according to the criteria of Fisher et al,[7] and the CT (Hounsfield) number of the SAH must be > 60.[8] Patients who do not satisfy all of these criteria may or may not be treated by cisternal drainage. Although it is not possible to predict which patients are at risk for developing vasospasm, the incidence is higher in patients with long-term residual SAH.

We performed intermittent irrigation with urokinase and ascorbic acid in patients whose postoperative CT scans documented poor clot washout following cisternal drainage. Because the primary SAH volume differed among patients treated by continuous irrigation, drainage alone, and intermittent irrigation, comparison of the treatment outcomes is of little value. Compared with the drainage-only group, the mean total blood volume drained in the intermittent irrigation group was higher and the mean value of the absorption spectrum of the oxyhemoglobin-specific peak at 576 nm was lower.

We posit that these results are attributable to the fibrinolytic effects of urokinase and the ability of ascorbic acid to decompose oxyhemoglobin. Compared with the continuous irrigation group, the drained blood volume in the intermittent irrigation group was lower and the absorption spectrum at 576 nm was higher. Although intermittent irrigation can be performed easily, it represents only a supplementary method to prevent vasospasm in patients with large residual SAH and those determined by postoperative CT scan to be at risk for vasospasm. Because we infuse irrigation fluid containing urokinase (120 IU/mL) and ascorbic acid (4 mg/mL) at a rate of 30 mL/hr, the daily delivery of urokinase and ascorbic acid is 86,400 IU and 2880 mg, respectively. The intermittent injection protocol delivers only 6000 IU of urokinase and 200 mg of ascorbic acid per day. Studies are under way in our laboratory to determine the appropriate urokinase and ascorbic acid concentrations for effective clot resolution and oxyhemoglobin breakdown and the optimal volume and frequency of irrigation.

Conclusion

We evaluated the ability of intermittent cisternal irrigation with urokinase and ascorbic acid to prevent vasospasm. Compared with cisternal drainage alone, intermittent irrigation removed a much greater amount of the clot. Analysis of the absorption spectrum of the drained fluid showed that intermittent irrigation effectively reduced the concentration of oxyhemoglobin. We are continuing to analyze the indications for administering intermittent irrigation in patients with SAH.

REFERENCES

1. Kodama N, Sasaki T, Kawakami M, Sato M, Asari J. Cisternal irrigation therapy with urokinase and ascorbic acid for prevention of vasospasm after aneurysmal subarachnoid hemorrhage: outcome in 217 patients. Surg Neurol 2000;53:110–118
2. Sasaki T, Kodama N, Kawakami M, et al. Urokinase cisternal irrigation therapy for prevention of symptomatic vasospasm after aneurysmal subarachnoid hemorrhage: a study of urokinase concentration and the fibrinolytic system. Stroke 2000;31: 1256–1262
3. Osaka K. Prolonged vasospasm produced by the break-down products of erythrocytes. J Neurosurg 1977;47:403–411
4. Kawakami M, Kodama N, Toda N. Suppression of the cerebral vasospastic actions of oxyhemoglobin by ascorbic acid. Neurosurgery 1991;28:33–40
5. Sato M. Prevention of cerebral vasospasm: experimental studies on the degradation of oxyhemoglobin by ascorbic acid. Fukushima J Med Sci 1987;33:55–70
6. Hunt WE, Kosnik EJ. Timing and perioperative care in intracranial aneurysm surgery. Clin Neurosurg 1974;21:79–89
7. Fisher CM, Kistler JR, Davis JM. Relation of cerebral vasospasm to subarachnoid hemorrhage visualized by computerized tomographic scanning. Neurosurgery 1980;6:1–9
8. Suzuki J, Komatsu S, Sato T, Sakurai Y. Correlation between CT findings and subsequent development of cerebral infarction due to vasospasm in subarachnoid hemorrhage. Acta Neurochir (Wien) 1980;55:63–70

Index

Note: Page numbers followed by *f* or *t* refer to figures and tables, respectively.